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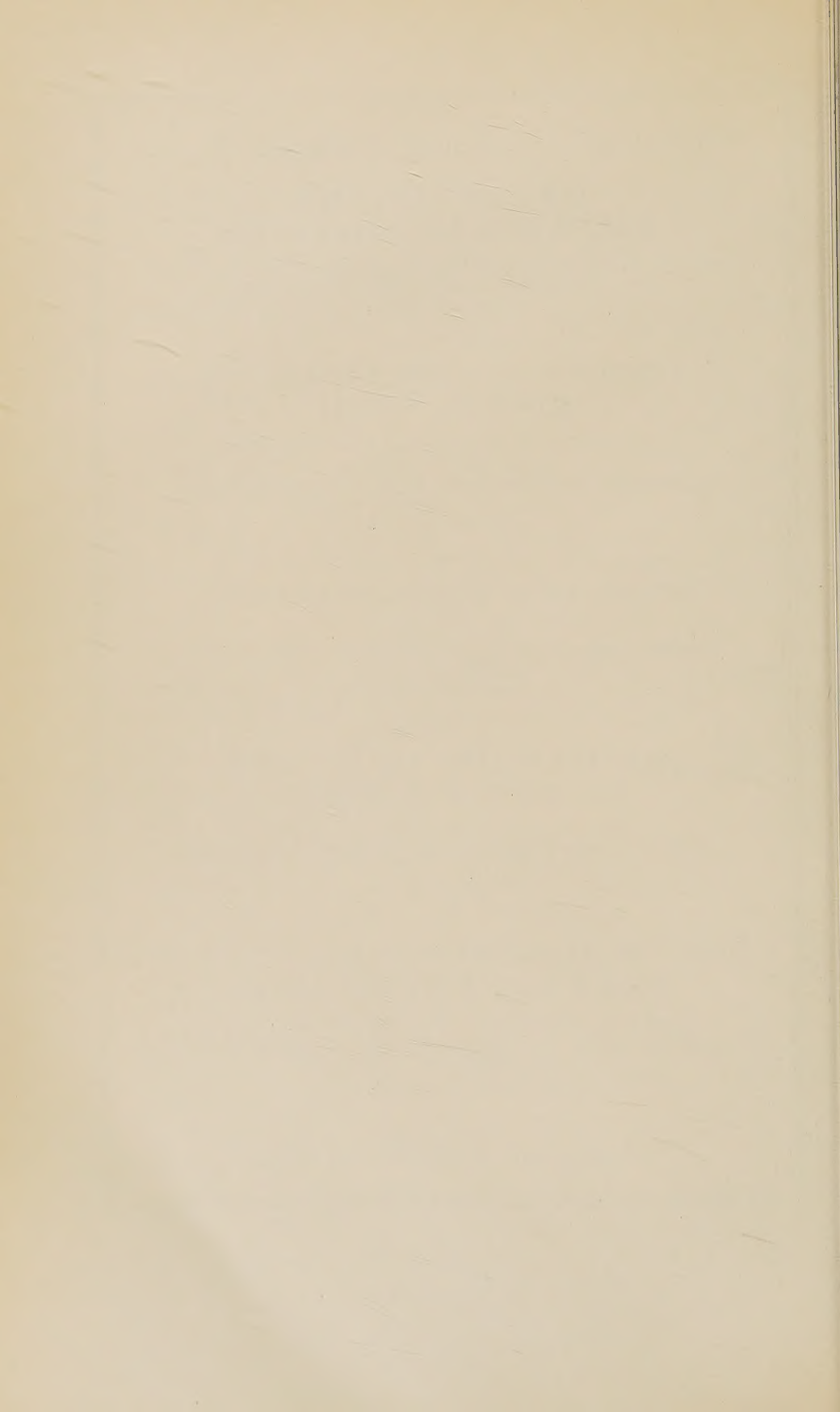
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CULTURAL STUDIES ON *ASCOCHYTA RABIEI* WITH SPECIAL REFERENCE TO ZONATION

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(With Plate 8)

Growth and zonation has been studied in three strains of *Ascochyta rabiei*, two of *A. pinodella* and one of *A. pisi*. For normal zonation in *A. rabiei* both the quantity and quality of the medium should be adequate for the production of a reasonable quantity of pycnidia throughout the growing period of the fungus. The effect of factors such as light, incubation temperatures, humidity and age of culture are discussed. Zones are produced when the fungus is incubated at alternating temperatures, whether it is kept in the light or in the dark. A zone is produced when the fungus is transferred to temperatures higher or lower than the optimum, because of a check in vegetative growth, which gives rise to higher pycnidial production in the marginal region reached by the fungus when it is transferred. The number of zones corresponds to the number of times the fungus is transferred to temperatures higher or lower than the optimum, and is n , $2n$ and $4n$ when the transfers are carried out after 48, 24 and 12 hr. respectively. Zones have also been produced artificially on seed pods of gram incubated at alternating temperatures. The production of concentric rings of pycnidia on gram pods under field conditions is probably due to daily fluctuations in atmospheric temperature during the period when infection takes place in nature.

The gram crop (*Cicer arietinum* L.) in the Punjab (Pakistan) is subject to blight disease caused by the fungus *Ascochyta rabiei* (Pass.) Labrousse. Brown spots of varying size appear on the stems, branches, leaf-stalks, leaflets and pods. The pycnidia of the fungus develop in concentric zones, on the pods and in artificial culture, and the investigation reported here was concerned with the factors responsible for such zoning. The work was carried out at the Imperial College of Science and Technology, London.

HISTORICAL

The commonest type of zonation in fungi is that in which bands of sporing mycelium alternate with bands which are sterile or spore less profusely. Brefeld (1877), MacDougall (1903) and Buller (1909), who studied environmental factors affecting sporulation in different fungi, concluded that spore production was either absent or very much reduced in darkness. Later, attention was drawn to zonation. Hedgecock (1906), Knischewsky (1909), Reidemeister (1909), Stevens and Hall (1909), Gallemmaerts (1911), Moreau (1912), Munk (1912), Coons (1916), Lehman (1923), Leonian (1924), Bisby (1925), Brown (1925) and Hall (1933) worked on a variety of fungi and concluded that the essential factors for the production of zones were the suitability of the culture medium and presence of alternate light and darkness. Ellis (1931), however, showed that zonation in *Pleospora herbarum* was brought about by temperature variation and not by alternation of light and darkness.

MATERIAL AND METHODS

The fungi included in the study were:

Two strains of *Ascochyta rabiei* isolated from diseased gram (*Cicer arietinum* L.) twigs brought from Lyallpur, Punjab, Pakistan (A.R. 1 and A.R. 2).

Ascochyta rabiei (Pass.) Labrousse, from the Centraalbureau voor Schimmcultures, Baarn, Holland (A.R.B.).

Ascochyta pinodella, isolated from diseased gram twigs (A.P.).

Ascochyta pinodella L.K. Jones, from Baarn (A.P.B.).

Ascochyta pisi Lib., stock culture kept in Plant Pathology Department, Imperial College of Science and Technology, London (*A. pisi*).

Work was first carried out with strains A.R. 1 and A.R. 2, and the other fungi were later included for comparative studies. Stock cultures were kept on oatmeal agar, and plates for detailed study were inoculated from spore suspensions in water. The following media were employed, the quantities given being the amounts in 1 l. of solution.

- (1) Oatmeal agar: oatmeal, 50 g.; agar, 15 g.
- (2) Gram meal agar: crushed gram seed, 50 g.; agar, 15 g.
- (3) Brown's agar: glucose, 2 g.; asparagin replaced by ammonium chloride, 2 g.; potassium phosphate, 1.25 g.; magnesium sulphate, 0.75 g.; agar, 15 g.
- (4) Brown's starch agar: medium (3) with 10 g. of potato starch added.
- (5) Potato agar: peeled potatoes, 200 g.; agar, 15 g.
- (6) Potato glucose agar: medium (5) with 15 g. of glucose.
- (7) Nutrient glucose agar: glucose, 20 g.; peptone 10 g.; beef-extract, 4 g.; sodium chloride, 5 g.; agar, 15 g.
- (8) Richard's agar: sucrose, 15 g.; potassium nitrate, 10 g.; agar, 15 g.

Relative pycnidial production and growth of the aerial mycelium are shown by + and - signs. The number of pycnidia was not generally counted, but on the basis of average counts in a few experiments the approximate pycnidial production per 5 sq.mm. in relation to the signs used was:

-	0-10	+++	57-80
+	11-25	++++	81-100
++	26-56	+++++	More than 100

Intermediate concentrations are represented. Thus, by + (+) is meant a concentration greater than + and less than ++. The following abbreviations are used in the tables: Pyc. for pycnidia, Zone for zonation, Diam. for colony diameter and Myc. for aerial mycelium. In all experiments treatments were carried out in triplicate.

EFFECT OF THE NUTRIENT MEDIUM ON GROWTH AND ZONATION

Experiment 1

Petri dishes, after inoculation with A.R. 1 and A.R. 2, were subjected to conditions of alternate light and darkness by placing them on the bench in the laboratory, where the temperature ranged between 15 and 18° C. The observations recorded after 18 days are given in Table 1.

Table 1. Growth features of A.R. 1 and A.R. 2 on eight different media

Medium	A.R. 1			A.R. 2		
	Diam. (mm.)	Myc.	Pyc.	Diam. (mm.)	Myc.	Pyc.
Oatmeal agar	45.0	—	++++	42.0	—	++++
Gram-meal agar	48.0	—	++++	46.0	—	++++
Brown's agar	28.0	—	++	27.0	—	++
Brown's starch agar	27.5	+	+++	27.0	+	+++
Potato agar	33.0	—	++	31.0	—	++
Potato glucose agar	29.0	++	++++	29.0	++	++++
Nutrient glucose agar	31.5	++	++++	30.0	++	++++
Richard's agar	27.0	++	++++	23.0	+	++++

Zones were not produced on any of the media used under conditions of alternate light and darkness. Media promoting intense pycnidial production were rejected for further studies on the presumption that zonation, if present, might be obscured. Nutrient glucose agar, however, was still retained in Exp. 2 for comparative purposes.

Experiment 2

Five media (potato agar, acidified potato agar, Brown's agar, acidified Brown's starch agar and nutrient glucose agar) were prepared and inoculated with A.R. 1 and A.R. 2. The dishes were divided into two groups, one being placed alternately at 25 and 15° C. for 24 hr. periods, and the other was kept on the laboratory bench at a temperature ranging between 15 and 18° C., but exposed to alternate light and darkness. The results recorded after 17 days showed that zones were not formed under the conditions of alternate light and darkness on the laboratory bench, but developed where the dishes were subjected to alternate temperatures of 25 and 15° C., showing that this was one factor responsible for the production of zones. The number of zones on different media, except nutrient glucose agar, was seven, irrespective of the different pH values, which ranged between 4.8 and 6.8. The work was now directed to determine the optimal medium for zonation. All the experiments described below were carried out under alternating temperatures of 25 and 15° C.

Experiment 3

Brown's starch agar was prepared and dishes were poured with 10, 20, 30 and 40 c.c. of the medium respectively. A.R. 1, A.R. 2 and A.R.B. were used. Observations recorded after 17 days showed that zones were clear and normal when the dishes were poured with 10 or 20 c.c. of the medium, but were not normal with increased quantities of the medium owing to higher mycelial and pycnidial production, resulting in obscuring and overlapping of zones. In further experiments, therefore, not more than 20 c.c. of medium was used per plate.

Experiment 4

Seven concentrations of Brown's starch agar ranging from 8N to N/8 (the medium which is normal being represented by N) were prepared. A.R. 1 was used, and observations recorded after 21 days showed that

N, N/2 and N/4 concentrations of the medium were suitable for the normal production of zones. These were underdeveloped at higher dilutions, while profuse development of mycelium obscured the zoning effect at higher concentrations. Moreover, at higher concentrations the linear colony growth decreased and symptoms of staling developed.

Experiment 5

Different percentages of various nitrogenous sources (asparagin, ammonium chloride, peptone or potassium nitrate) were used in the preparation of Brown's starch agar. Observations recorded 19 days after inoculation showed that although the omission of nitrogen from the medium reduced the number of pycnidia it did not affect the linear colony diameter and the number of zones. Increase in the amount of nitrogen increased pycnidial production, but the zones were overlapping when the concentration was increased beyond 0.05 % potassium nitrate, 0.2 % ammonium chloride, 0.3 % asparagin and 0.5 % peptone.

Experiment 6

The carbohydrate constituents of the medium, i.e. glucose and starch, were mixed in different proportions to give twenty-four media having various C/N ratios, the carbon being in the form of glucose, starch or both. A.R. 1 was used, and the results 21 days after inoculation showed that the omission of carbohydrate from the medium not only affected linear colony growth, aerial mycelium and pycnidial production, but also repressed the development of zones. When either of the carbohydrates was omitted zones were produced, but they were not well defined in the absence of starch owing to the decrease in the number of pycnidia produced. The development of zones was normal within the range 0.1–0.5 % glucose, but further increase produced a thick-lobed growth, which ultimately staled and thus reduced the number of zones. Starch, when increased beyond 0.5 % in a medium without glucose, produced a similar effect, but it could be increased even up to 1.0 % without affecting zonation, provided 0.2 % glucose was also present in the medium.

Experiment 7

The glucose of Brown's starch agar was replaced by equal quantities of maltose, lactose and sucrose. The results, after 19 days' growth of A.R. 1, showed that all the sugars used behaved in a very similar manner.

Experiment 8

The tribasic potassium phosphate of Brown's medium was replaced by potassium mono-hydrogen phosphate or potassium di-hydrogen phosphate. Media containing different percentages of these salts were prepared together with one containing none. The results showed that both quality and quantity of phosphates affected pycnidial production and also zonation. Pycnidial production increased with increase in tribasic potassium phosphate and potassium mono-hydrogen phosphate, and zones began to overlap when the quantity was increased beyond 0.15 %. On the other hand,

pycnidial production was reduced when the percentage of potassium di-hydrogen phosphate was beyond 0.037 %.

Experiment 9

Brown's starch agar was prepared with six concentrations of magnesium sulphate ranging between 0 and 0.2 %. A.R. 1 was used, and after 21 days no difference in cultural features and zonation could be distinguished.

Experiment 10

The initial pH of Brown's starch agar was adjusted to various values by the addition of decinormal hydrochloric acid or sodium hydroxide. Plates were inoculated with A.R. 1 and the observations recorded after 17 days are given in Table 2.

Table 2. *Effect of hydrogen-ion-concentration of the medium on zonation*

Initial pH	Diam.	Myc.	Pyc.	Zone	Remarks
8.2	29.6	—	++	7	Zones not clear
7.4	31.7	—	+++	7	Zones not clear
6.8	32.0	+	+++	7	Zones clear
6.2	36.1	+	+++	7	Zones clear
3.6	30.0	+	++(+)	7	Zones clear
4.2	27.6	++	++	7	Zones clear
4.0	21.5	++(+)	+	3	Pyc. only in centre
2.8	14.6	++++	—	Nil	Thick carpet of mycelium
2.4	12.3	++++	—	Nil	Thick carpet of mycelium

The following points are clear from the results:

(i) With increase in the acidity of the medium mycelial production increases, but at a pH lower than 6.0 there is a steady decrease in colony diameter and number of pycnidia. No pycnidia are produced below pH 2.8.

(ii) The region of normal zonation is pH 4.2–6.8. Zones do not develop in higher acidities owing to profuse development of mycelium. In media with pH values above 6.8 they are not distinct, thus indicating that an acidic medium is essential for the development of clear zones.

Similar results were obtained when potassium di-hydrogen phosphate or glucose were used in higher concentrations on account of development of similar pH values.

EFFECT OF LIGHT ON GROWTH AND ZONATION

Experiment 1

Potato agar was prepared, and after inoculation with A.R. 1 and A.R. 2, the dishes were divided into six sets. Two were kept on the bench at 15–18° C., one in the dark, and the other under alternate light and darkness. The third and the fourth sets were placed at 25° C.—one in constant darkness and the other in constant light. The remaining two were kept alternately for 24 hr. at 25 and 15° C., one of them being in the dark and the other under alternate light and darkness. The results recorded after two days showed that:

(i) There was no difference in cultural features of the fungi whether grown under constant light, constant darkness, or alternate light and darkness at constant temperatures, and no zones were produced.

(ii) Zones were produced only in the dishes, placed alternately for 24 hr. at 25 and 15° C., irrespective of presence or absence of light (see Pl. 8, figs. 1-6.).

(iii) A.R. 1 showed a tendency to zonation in dishes kept under alternate light and darkness or constant darkness on the bench. This may be due to its greater sensitiveness to small fluctuations (15-18° C.) in daily temperatures.

Experiment 2

The dishes after inoculation with A.R. 1 and A.R. 2 were kept under constant darkness for five days on the bench, when the growing edge of the fungus was marked. They were then exposed to daylight for eight hours. On every third day the same exposure was given and this treatment was repeated four times. No zones were produced.

EFFECT OF TEMPERATURE ON GROWTH AND ZONATION

Experiment 1

Cultures of A.R. 1 and A.R. 2 were incubated for 24 hr. at 25° C. and alternately at one of the following temperatures: 10, 15, 20, 30 or 35° C. Results after 19 days showed that zonation was normal except when incubated alternately at 25 and 35° C., growth then being checked at 35° C. It will be shown later that zones can be made to develop even in this combination by reducing the period of incubation at 35° C. The results were similar for A.R. 2, except that zones were not clear when the difference between the two incubation temperatures was only 5° C.

Experiment 2

In this experiment 15° C. was maintained as the basic temperature and was alternated with temperatures of 10, 20, 25, 30 or 35° C. Zones were normal in all combinations other than 15-10 and 15-35° C., when growth of the fungus was either checked or much restricted because one of the temperatures in these combinations was unfavourable. The zones were less clear at 15-20° C., but were clear when the temperatures were 20-25° C., indicating that the factor responsible for zonation is the rate of difference in growths at alternate temperatures of incubation.

Experiment 3

The previous experiments had shown that a zone was produced each time with the transfer of the culture from 25 to 15° C. The effect of keeping the dishes for different periods at 25 and 15° C. was then studied. Seven combinations were arranged. The dishes, after inoculation with A.R. 1, A.R. 2 and A.R.B. were kept at 25° C. for the first 72 hr. and then regularly alternated between 15 and 25° C. according to the scheme given in Table 3, which embodies the results recorded after 19 days (see also Pl. 8, figs. 7-14).

The results showed that the number of zones was 14, 8 and 4 when transferences to 15° C. occurred daily, every 2 days and every 4 days respectively. The zones were short by two in the first combination because

they fused together in the central area on account of their close formation. Similarly, the number of zones in other combinations was equal to the number of times the dishes were transferred to 15° C.

Table 3. *Effect of different durations of incubation at 25 and 15° C.*

Treatment	A.R. 1		A.R. 2		A.R.B.	
	Diam.	Zones	Diam.	Zones	Diam.	Zones
(1) 12 hr. at 25° C. and 12 hr. at 15° C.	33·5	14	31·8	14	57·6	14
(2) 12 hr. at 25° C. and 24 hr. at 15° C.	35·4	11	29·5	11	46·4	11
(3) 12 hr. at 25° C. and 48 hr. at 15° C.	38·4	7	25·3	7	45·8	7
(4) 24 hr. at 25° C. and 24 hr. at 15° C.	36·3	8	35·3	8	42·8	8
(5) 24 hr. at 25° C. and 12 hr. at 15° C.	44·8	11	44·3	11	64·4	11
(6) 48 hr. at 25° C. and 12 hr. at 15° C.	46·1	7	39·6	7	61·1	7
(7) 48 hr. at 25° C. and 48 hr. at 15° C.	42·8	4	36·3	4	45·1	4

Experiment 4

The effects of incubating the fungus at constant and alternate temperatures at different stages of its growth were then investigated. The plates after inoculation with A.R. 1, A.R. 2 and A.R.B. were divided into the following four sets:

- Kept initially at constant temperature, then at alternate temperatures, again at constant temperature and finally at alternate temperatures.
- Kept initially at alternate temperatures and then at a constant temperature.
- Kept initially at alternate temperatures, then at constant temperature and finally at alternate temperatures.
- Kept initially at constant temperature and then at alternate temperatures.

The plates were thus variably treated regarding stage, time and number of transfers given at alternate temperatures. Zones were produced whenever the plates were incubated at alternate temperatures, irrespective of the stage of the growth of the fungus, and they ceased to appear when the temperature was kept constant (see Pl. 8, figs. 15-17). However, the zones produced in the later stage of growth were not clear because of reduction in pycnidial formation which normally occurred with the continued growth of the fungus, even in control plates.

Experiment 5

To find the minimum period that the fungus must be kept at alternate temperatures in order that a zone may develop, an experiment was arranged in which the plates, after 5 days' incubation at 25° C., were placed for either 1, 2, 4 or 6 hr. at 5, 15, 20, 30 and 35° C. Ten alternations of incubation temperatures were given, and the results showed that the normal number of zones developed when the dishes were placed for even 1 hr. at 5, 30 and 35° C., while at least 6 hr. were necessary at 15 and 20° C. The shorter interval is probably related to more severe checks to growth at the temperatures concerned.

EFFECT OF HUMIDITY ON GROWTH AND ZONATION

Three days after inoculation with A.R. 1 dishes were divided into three sets. The first and the second were kept continuously in an atmosphere of 0 and 100 % humidity respectively, while the third was alternately placed for 48 hr. under these two humidities. The experiment was run at a constant temperature of 25° C. Zones did not develop at all, but there were some differences in linear growth, and pycnidial production was highest, lowest and intermediate at constant low humidity, constant high humidity and alternate humidities respectively.

EFFECT OF AGE OF CULTURE ON GROWTH AND ZONATION

The previous experiments had shown that the number of zones was equal to the number of times the dishes were transferred to 15° C., but there was no development of zones if such transfers were carried out within 3 days of inoculation. The age of the culture, ranging from 7 to 57 days, had no effect on the production of the first zone. The first zone could be developed only in those dishes in which transfer was made 72 hr. after inoculation; the other dishes showed a central region about 8 mm. in diameter, equal to 3 days' growth of the fungus at 25° C., as unzoned and packed with pycnidia. By further experiment it was found that the first zone could be made to develop when the medium used lacked a source of nitrogen, because this reduced pycnidial production in the centre. Again, when the fungus was grown on such a medium, the first zone could develop when the transfer was made even after 24 hr. of inoculation, thus indicating that higher pycnidial production in the centre had been responsible for the loss of identity of the first zone.

ZONATION IN OTHER FUNGI

Some experiments were carried out to study zonation in *Ascochyta pisi* and in the two strains of *A. pinodella*, designated A.P. and A.P.B. The results showed that the colony growth of A.P. and A.P.B. was higher under conditions of alternate light and darkness, but pycnidial production in A.P. was higher under constant darkness than under alternate light and darkness. Zones were produced in A.P. under alternating temperatures of incubation, and there was also a great tendency for zonation under alternate light and darkness. In *A. pisi* and A.P.B. only mycelial types of zones were produced under alternating temperatures of incubation.

ZONATION ON SEED PODS OF THE GRAM PLANT

Detached green gram pods, surface-sterilized with mercuric chloride, were inoculated with drops of spore suspension of A.R. 1 and incubated for 3 days in moist chambers at 25° C. They were then divided into three sets—one being kept continuously at 25° C., the second exposed to light and darkness on the laboratory bench at approximately 15–18° C., and the third kept for 24 hrs. alternately at 25 and 15° C. The results were recorded on the eleventh day after inoculation, thus giving the third set four transfers between 15 and 25° C.

In the first two sets, which were kept at almost constant temperature, pycnidia were scattered over the surface of the pods, while on those which had been incubated at alternating temperatures of 15 and 25° C. the pycnidia were arranged in concentric zones. The number of zones was three and not four as expected. This was probably due to under-development of the last zone at the time of recording observations. Rotting of the pods prevented observations after 11 days. Alternation of incubation temperatures is thus responsible for the zonal arrangement of pycnidia on the surface of gram pods in the laboratory.

The record of field temperatures at Lyllapur shows that the daily range of difference in maximum and minimum temperatures (7.7–17.2° C. in February and 10.0–18.3° C. in March) at the time when the disease usually appears on the pods is sufficiently wide to account for the production of zones under natural conditions.

DISCUSSION

An analysis of the factors responsible for the production of zones in *Ascochyta rabiei* in the laboratory indicates that both quality and quantity of the medium are important. The medium should be neither too rich nor too ample to give excessive mycelial growth and sporulation, and neither too poor nor too small in quantity to produce feeble growth. Under the first-named conditions the zones remain obscure because of overlapping arising from intense mycelial or pycnidial production; and under the other conditions the zones are incomplete on account of under-development of pycnidia. When the quantity and quality of a medium is suitable, the other important factor for the production of zones is variation in temperature during incubation. When the fungus is transferred to temperatures higher or lower than the optimum, more pycnidia are produced in the region to which its growing margin extended at the time of transfer from one temperature to another, thus giving a pycnidial zone. Similarly, under laboratory conditions of alternating temperatures, zones are produced on pods of gram plants, and it seems probable that the same factor is responsible for zone production under natural conditions, for the daily variations experienced in areas where gram is grown are of the same order as those required to produce zones under laboratory conditions.

In the literature different workers have emphasized that the medium should be such as to be capable of producing an unstaled type of growth during the growing period. Alternation of light and darkness or variation in the temperature of incubation is also necessary.

Broadly speaking zoning fungi can be divided into two main groups—one producing zones of sporulation type and the other producing zones of mycelial type. The fungi which produce zones of sporulation type can be further divided into those which produce their spores either in the light* only or in the dark† only, or in both (but with an increased tendency to

* *Fusarium discolor sulphureum*, *Plenodomus fuscomaculans*, *Sclerotinia laxa*, *Penicillium luteum*, *Diaporthe soyae*. Some members of the Sphaeropsidales.

† *Cephalothecium roseum*, *Mucor* sp., *Penicillium* sp.

sporulate in one of them*); and those† which are capable of producing spores both in the light and the dark without showing any appreciable increase or decrease in either case. The fungi which belong to the first three types generally produce zones under conditions of alternate light and darkness, and those which belong to the fourth type are not affected by this change but may produce zones with variation in incubation temperatures. Fungi belonging to the first three types may also differ from each other in their need for different periods of exposure to light or darkness before they are capable of producing definite zones. Fungi which belong to the fourth type may also show differences in their requirements as to the minimum range of variation in incubation temperatures and the time over which they should be kept at a particular temperature for the normal development of zones.

Fungi belonging to the second main group which do not freely sporulate may, when placed alternately at two temperatures, show zonation of mycelial type representing the two types of mycelial growth characteristic of the two temperatures chosen.

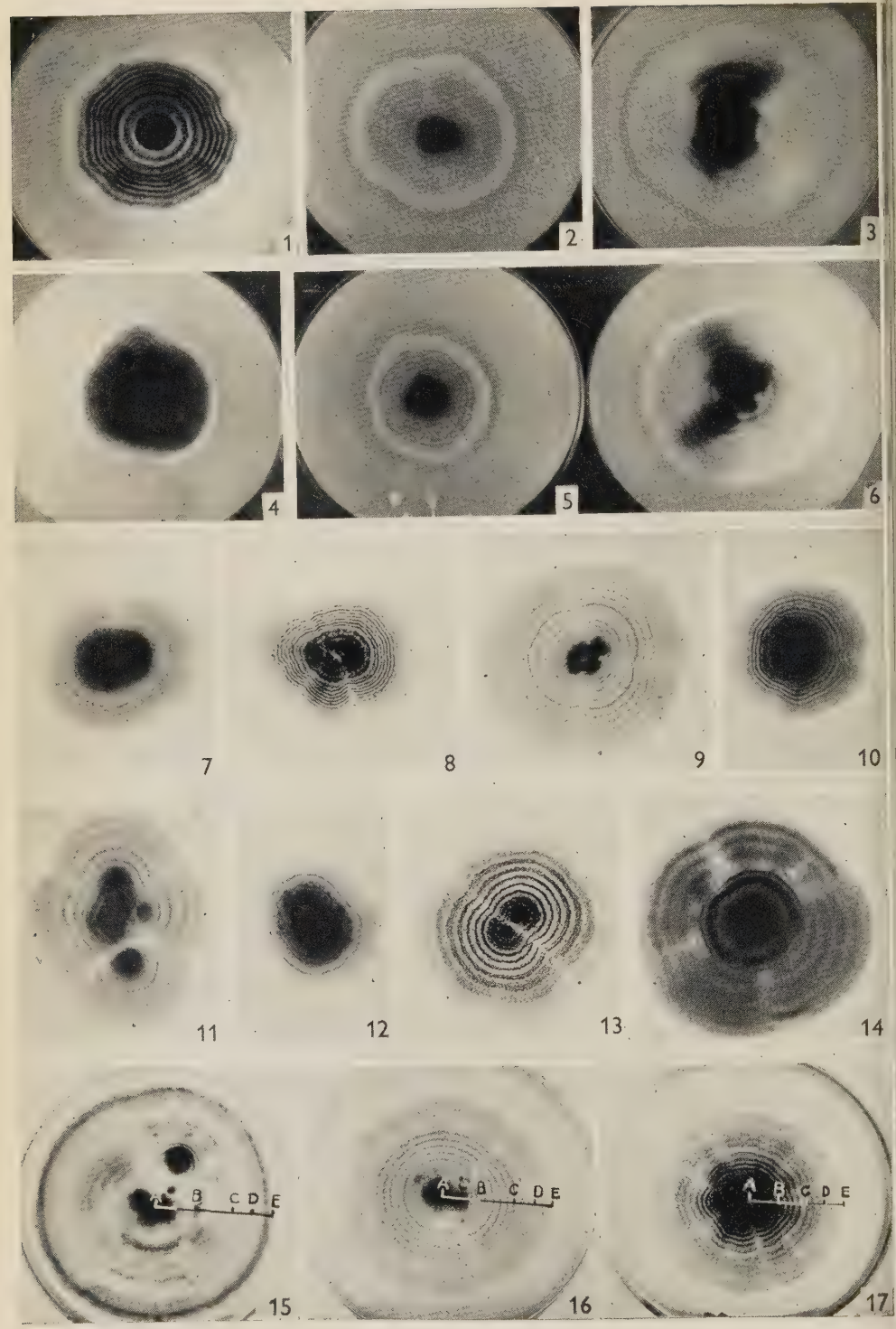
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* *Sclerotinia fructigena* (sporulation more in the light), *S. fructicola* (sporulation more in the dark), *Botrytis cinerea* (sporulation more in the light).

† *Pleospora herbarum*.



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EXPLANATION OF PLATE 8

Effect of light and darkness on zonation.

- | | | |
|----------------|---|---|
| Fig. 1. A.R. 1 | } | Kept constantly in the dark but at alternating temperatures of 25° and 15° C. |
| Fig. 2. A.R. 2 | | |
| Fig. 3. A.R.B. | | |
| Fig. 4. A.R. 1 | } | Kept under light at 15° C. and darkness at 25° C. alternately. |
| Fig. 5. A.R. 2 | | |
| Fig. 6. A.R.B. | | |

Effect of different durations of incubation at 25° and 15° C. on zonation.

- | | | |
|-----------------|---|--|
| Fig. 7. A.R. 2 | } | Kept for 12 hr. at 25° C. and 12 hr. at 15° C. |
| Fig. 8. A.R. 1 | | |
| Fig. 9. A.R. 2 | } | Kept for 12 hr. at 25° C. and 24 hr. at 15° C. |
| Fig. 10. A.R. 1 | | |
| Fig. 11. A.R. 1 | | |
| Fig. 12. A.R. 2 | } | Kept for 12 hr. at 25° C. and 48 hr. at 15° C. |
| Fig. 13. A.R. 1 | | |
| Fig. 14. A.R.B. | | |

Effect of alternation of incubation temperatures at different stages of growth of the fungus on zonation.

- | | | |
|-----------------|---|---|
| Fig. 15. A.R.B. | } | Kept initially at constant temperature, then at alternating temperatures, again at constant temperature and finally ending with alternating temperatures. |
| Fig. 16. A.R. 2 | | |
| Fig. 17. A.R. 1 | | |

(Accepted for publication 3 October 1951)

A NEW SPECIES OF HYPHOMYCETE ATTACKING THE STEM EELWORM *DITYLENCHUS DIPSACI*

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(With 1 Text-figure)

Ditylenchus dipsaci, found in diseased *Calceolaria*, was attacked by *Verticillium sphaerosporum* n.sp. with sticky spores adhering to the nematode cuticle. The fungus penetrated the nematodes, killed them, and produced fertile hyphae outside the host bearing phialides singly or in small groups, from which bunches of spherical spores were produced on apical sterigmata.

Ditylenchus dipsaci (Kühn, 1857), Filipjev, 1936, has twice been recorded by T. Goodey (1929, 1938), causing disease in *Calceolaria* sp. On the second occasion he found that some of the nematodes were attacked by *Arthrobotrys oligospora* Fres., a hyphomycete which catches nematodes by means of sticky, looped hyphae.

In September 1949 some diseased plants of a garden variety of *Calceolaria integrifolia* L., in which the stem eelworm *Ditylenchus dipsaci* was present, were sent to this Department for confirmation of the identity of the nematode. The material had come from the Kilmarnock Parks Department, and the eelworms were causing a soft black stem rot and black necrotic leaf lesions. Dr H. F. Dovaston, who sent the material, had also found *Phytophthora cryptogea* in it.

Examination of the nematodes, after teasing the diseased material in water, showed that in this instance also many were attacked by a fungus. The nematodes were covered to a varying extent with fungus spores adhering to the cuticle. Other nematodes were found with hyphae growing from the body. Reference to Dollfus (1946) indicated that the fungus was probably a species of *Acrostalagmus* or *Cephalosporium*, but it did not tally exactly with the species there described. Specimens were therefore submitted to Dr C. L. Duddington for an opinion. His view was that although the fungus had certain resemblances to *Acrostalagmus obovatus* (Drechsler, 1941) it was not identical, and that it was probably an undescribed species best referred to the genus *Verticillium*.

The mode of attack on the nematodes appears to be as follows. As the nematodes move about in the diseased plant tissue they come into contact with the spores which have sticky coats. The spores adhere, sometimes in ones or twos, sometimes in great numbers, to all parts of the nematode cuticle (Fig. 1 A). Each spore develops a hypha which penetrates the cuticle by a minute hole, and then widens inside the eelworm as a hypha about $1.6\text{--}4\mu$ wide with septa $5\text{--}16\mu$ apart (Fig. 1 B). The hyphae branch as they grow, so that eventually the whole body becomes filled with assimilative mycelium, and the body contents are no longer visible. Next fertile hyphae grow out from the body, again through small perforations in the

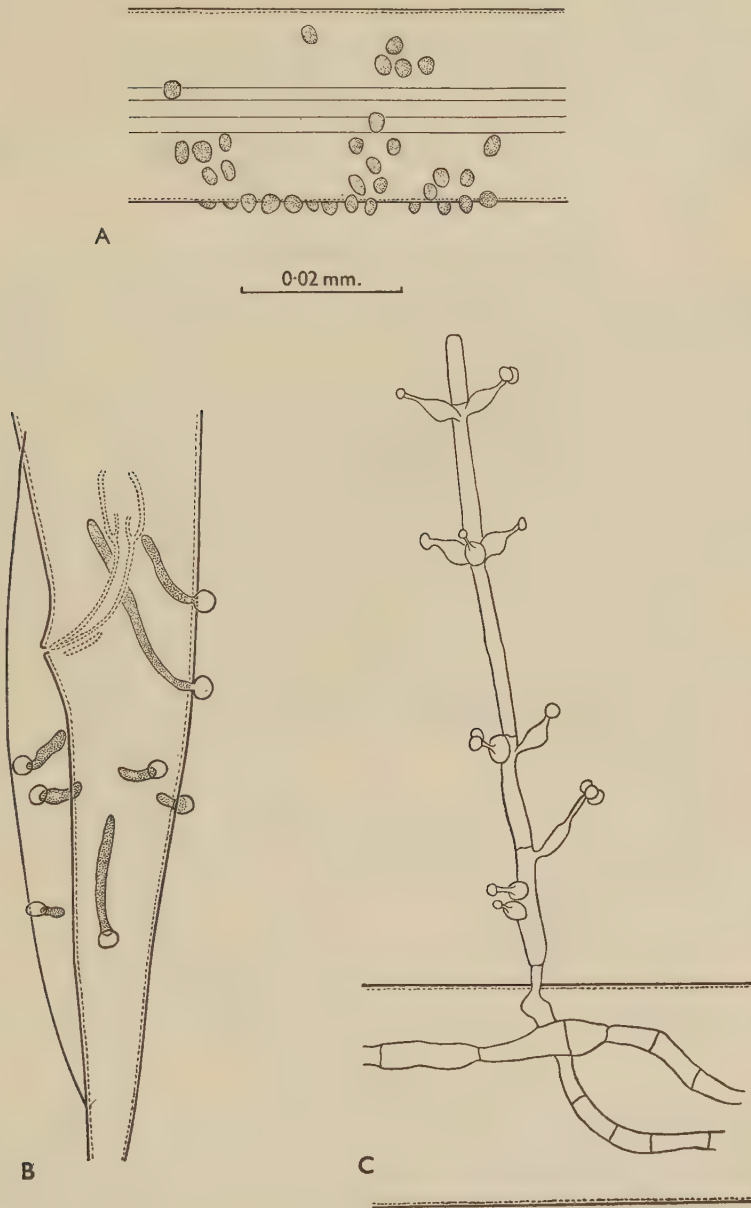


Fig. 1. A, part of *Ditylenchus dipsaci* in lateral view with adhering spores; B, part of male tail of *D. dipsaci* showing germinating spores in various stages; C, part of *D. dipsaci* with ramifying internal hyphae and an extruded fertile hypha bearing phialides.

body wall. These hyphae carry flask-shaped phialides near the distal ends of the cells, either singly or in whorl-like groups of up to three or four (Fig. 1 C). They bear spherical, sessile spores, $2-3\ \mu$ in diameter in bunches on terminal sterigmata. The spores are very easily dislodged so that in stained preparations only one or two are seen on each phialide.

Since the fungus appears not to have been encountered previously it is accordingly described as a new species of the genus *Verticillium* with the specific epithet *sphaerosporum*, from the form of the spores.

Verticillium sphaerosporum sp. nov.

Mycelium nutritum hyalinum, ramosum, septatum, septis $5-16\ \mu$ inter se distantibus, intra nematoda viventia evolutum, ex hyphis filiformibus $1.5-4\ \mu$ latis constans. Rami fecundi extra nematodum emortuum evoluti, hyalini; axibus $30-200\ \mu$ longis, circa $1.5\ \mu$ latis, septatis, cellulis $8-25\ \mu$ longis $1-4$ phialides a fronte ferentibus. Phialides lageniformes, $5-8\ \mu$ longae, in max. diam. $2-2.5\ \mu$ latae, sterigma terminale circa $0.5\ \mu$ latum ferentes. Pleraque conidia sessilia, hyalina, globosa, $2-3\ \mu$ in diam., cuiusque in apice sterigmati pariuntur, ita quasi glutine inducta ut nematodi cuticulae facile adhaereant.

Inveniebatur ut hyperparasitus, perimens *Ditylenchum dipsaci* (Kühn, 1857) Filipjev, 1936 qui *Calceolarium integrifolium* L. Kilmarnockiensem morbo afficiebat.

Assimilative mycelium' hyaline, branched, septate, the septa $5-16\ \mu$ apart, growing within living nematodes and composed of filamentous hyphae $1.5-4\ \mu$ wide. Fertile branches formed outside the dead host, hyaline, the axial filament $30-200\ \mu$ long, about $1.5\ \mu$ wide, septate, the cells $8-25\ \mu$ long bearing one to four phialides at the distal end. Phialides flask-shaped, $5-8\ \mu$ long, $2-2.5\ \mu$ wide at the greatest diameter, bearing a distal sterigma about $0.5\ \mu$ wide. Numerous sessile, hyaline, spherical conidia $2-3\ \mu$ in diameter produced at the apex of each sterigma. Conidia coated with a sticky substance so that they readily adhere to the host nematode cuticle.

Found as a hyper-parasite destroying *Ditylenchus dipsaci* (Kühn, 1857), Filipjev, 1936, which was causing disease in *Calceolaria integrifolia* from Kilmarnock, Scotland.

Slides of this fungus are in the author's collection at Rothamsted Experimental Station, Harpenden.

The author wishes to thank Dr C. L. Duddington for his help in matters of identification and Mr D. H. Boalch for his assistance in preparation of the Latin diagnosis.

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(Accepted for publication 16 October 1950)

CALOTHYRIUM PINASTRI

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(With 1 Text-figure)

Calothyrium pinastri (Fuckel) Theissen, a member of the Microthyriaceae new to Great Britain, is figured and redescribed, and attention is drawn to the presence of an internal mycelium linked with the external mat by way of the stomata.

On 28 September 1950, Dr R. W. G. Dennis, of the Royal Botanic Gardens, Kew, found *Calothyrium pinastri* (Fuckel) Theissen on dying leaves of *Pinus sylvestris* at the head of Loch Morlich, Glenmore, Rothiemurchus, Inverness-shire. There appear to be no previous records of the occurrence of this fungus in Great Britain. Fuckel, in his *Symbolae Mycologicae*, Nachtrag, 3, 1877, p. 29, called this fungus *Microthyrium pinastri*, and gave the

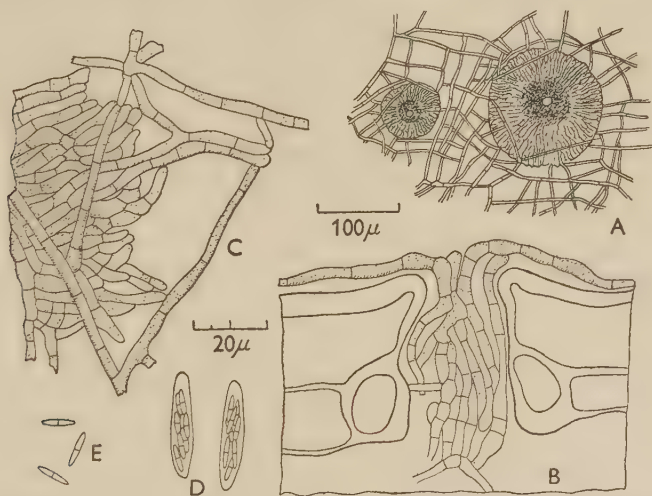


Fig. 1. *Calothyrium pinastri*. A, Superficial mycelium with thyriothecia. B, Hyphae passing through a stoma. C, Margin of thyriothecium. D, Asci. E, Spores.

following brief description. 'Scutellis ascigeris sparsis, superficialibus, spermogonia triplo quadruplove minoribus, atris, umbilicatis; ascis oblongis, fasciculatis, sessilibus, 22 Mik. long., 4-5 Mik. crass, 8 sporis; sporidia matura, eliberata nondum vidi.' Höhnelt, in his *Fragmente zur Mykologie*, no. 518, 1910, drew attention to the presence of superficial mycelium without hyphopodia, gave spore measurements, and referred the associated pycnidia to a new genus, *Sirothyriella*. Theissen made the transfer to *Calothyrium* in *Öst. bot. Z.* **62**, 219, 1912.

Examination of fresh material has shown that, besides the external mycelium, there are hyphae embedded in the tissues of the leaf, and that these link up with the superficial mat by way of the stomata. Hansford, in *Mycol. Pap. Commonw. Mycol. Inst.* no. 15, p. 179, 1946, states that in *Calothyrium jahnii* Syd. the external mycelium penetrates the cuticle in places and forms small patches of hyaline mycelium beneath the cuticle and covering a few cells of the epidermis, and that in *C. ryani* Stev. it is connected at intervals with subcuticular vesicles, each of which produces a single very fine hypha which penetrates between the cells of the epidermis and becomes wider and septate in the intercellular spaces of the mesophyll. In *C. pinastri* there is an extensive superficial mycelial mat made up of a network of branched and anastomosing, olive to pale brown, septate hyphae $2-3\mu$ thick (Fig. 1 A). Hyphae in the stomata (Fig. 1 B) are pale brown, and in the substomatal cavity they are subhyaline and sometimes slightly swollen (to 5μ); in the leaf tissues they are hyaline and $1.5-3\mu$ thick. The thyriothecia (Fig. 1 A) are scattered, round, brown, $100-150\mu$ in diameter, with an ostiole $8-10\mu$ wide; the upper wall is composed of wavy, radiating hyphae $2-4\mu$ thick (Fig. 1 C). The asci (Fig. 1 D) are cylindrical to clavate, $18-30\mu$ long, $4-6\mu$ wide, 8-spored with the spores usually in two rows. The ascospores (Fig. 1 E) are hyaline, 1-septate, $8-10\mu$ long and $1.5-2\mu$ wide in the middle. There are no paraphyses. No pycnidia were seen; Höhnelt described them as being similar to the thyriothecia but larger ($250-400\mu$), with the conidiophores formed on the underside of the covering membrane and bearing hyaline, non-septate conidia $6-7\mu$ long and 1μ thick.

(Accepted for publication 19 October 1950)

HELICODENDRON GIGANTEUM N.SP. AND OTHER AERIAL-SPORING HYPHOMYCETES OF SUBMERGED DEAD LEAVES

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(With Plate 9 and 4 Text-figures)

The fungus flora of leaves decaying at the bottom of ponds and lakes, where the water is more or less static, is made up largely of Hyphomycetes (mostly helico-sporous) which sporulate only when the leaves are exposed to air. A number of these fungi have been grown in pure culture, and five have been identified as *Helicoon fuscosporum* Linder, *Helicodendron tubulosum* (Riess) Linder, *H. triglitzensis* (Jaap) Linder, *H. hyalinum* Linder; and *Clathrosphaera spirifera* Zalewski.

Helicodendron giganteum n.sp. is described in detail.

INTRODUCTION

Decaying leaves, belonging to various species of tree, dredged from the bottom of static ponds, where conditions are probably more or less anaerobic, have a characteristic black appearance. If such leaves are rinsed in water and kept damp, but not submerged, in Petri dishes exposed to light for a few days, white or yellow glistening spots frequently appear on the exposed surface. These, when examined under the microscope, are seen to consist of clusters of spores, mostly of a helicoid form. A striking feature is that each spore or spore group is apparently unwettable and air is trapped in its coils, so that if it is temporarily forced below the water it soon bobs up to the surface again. If a leaf bearing spores is submerged in water the ripe conidia float off on the surface. It seems that in the pond the fungus is present in the leaf as a mycelium, and that only when exposed to air do spores develop. The regularity with which these fungi occur on such leaves was first noticed by Dr H. M. Canter (Mrs J. Lund) in collections from Windermere. Fungi of the same type have since been found on submerged leaves, twigs and bark of trees collected from localities in and around London, including Chislehurst, Oxted, Swanley, Guildford, Hammer, Waggoners Wells (Hindhead), Wisley and Hampstead Heath.

Eleven species of these fungi have so far been recognized and isolated in pure culture on 2% malt agar. Colonies of most species sporulate freely after exposure to daylight for a few days. Five have so far been identified as *Helicoon fuscosporum* Linder, *Helicodendron tubulosum* (Riess) Linder, *H. triglitzensis* (Jaap) Linder, *H. hyalinum* Linder, and *Clathrosphaera spirifera* Zalewski. Only the clathroid spores described by Zalewski (1888) belong to *C. spirifera*, the helicoid spores which he also ascribed to this species are probably those of a species of *Helicodendron* (see van Beverwijk, 1951). Of the remaining six isolates, one is clearly a new species of *Helicodendron*, and is the subject of this paper.

The helicosporous Hyphomycetes are by no means limited to submerged leaves decaying under relatively anaerobic conditions, although they apparently form the characteristic flora of such leaves. Linder (1929), whose monograph on helicosporous Fungi Imperfecti is the starting point of modern work on these fungi, has reported species of *Helicodendron* and *Helicoon* from decaying wood, but always from material that had been very damp.

***Helicodendron giganteum* sp. nov.**

Fungus aquaticus mycelio repente ramoso septato. Conidium filiforme, septatum, in tubulum ovatum vicibus 20 (vel paucioribus) convolutum, longum ad 200μ , latum ad 70μ , hyalinum vel brunneum. Ex quoque conidio alia conidia saepe oriuntur.

Hab. In foliis putrescentibus *Fagi* et *Quercus*, Windermere, Anglia.

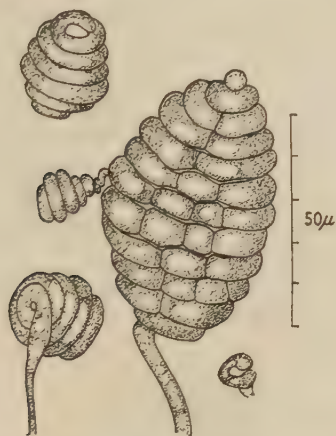
Aquatic fungus with branched septate mycelium. Conidium hyaline to brown, up to $200 \times 70\mu$, consisting of a septate hypha coiled to form a three-dimensional helix of up to twenty coils. Each spore may proliferate to form secondary conidia.

On submerged decaying leaves of beech and oak, Windermere.

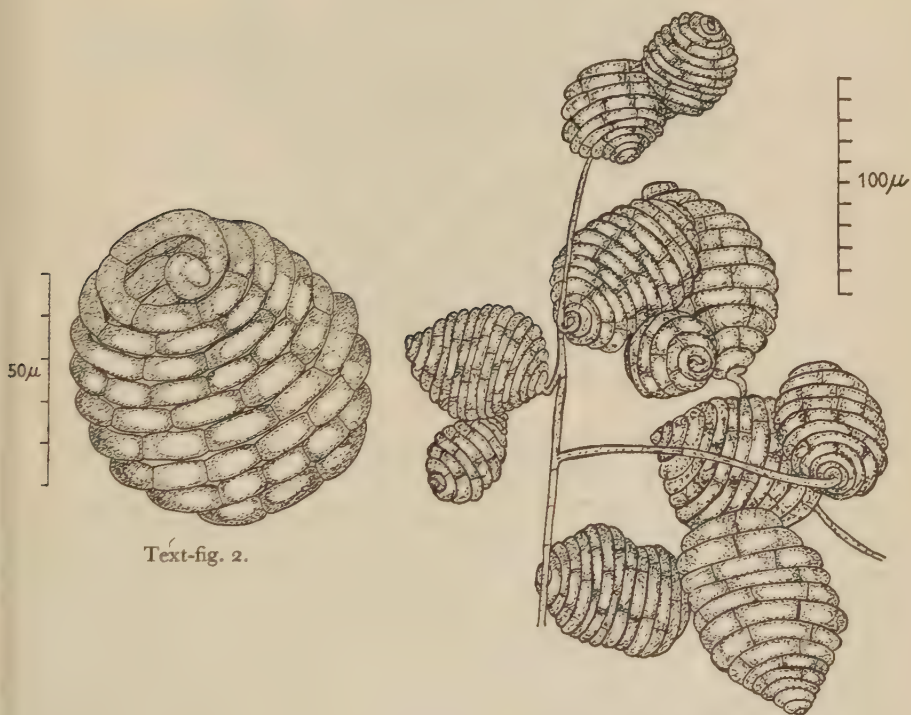
A culture of the fungus has been sent for incorporation in the collection of cultures at the Commonwealth Mycological Institute, Kew, and the type specimen has been deposited in Herb. C.M.I., no. 44159.

This fungus has been found on one occasion only on decaying oak and beech leaves collected from Windermere in January 1949. Clusters of tightly coiled spores appeared on the surface of these leaves after exposure for a few days to light in a moist atmosphere (Pl. 9, fig. 1).

The spores are formed terminally and laterally on long (300μ or more) simple or branched conidiophores $2-5\mu$ thick. Observations of stages in development indicate that at first a tightly coiled helix is formed by the apical growth of the conidial filament, which is initially about 3μ thick. Subsequent enlargement of the spore is brought about by increase in thickness of the filament and also by its continued apical growth (Text-figs. 1, 2). Each mature spore consists of a many-septate filament up to about 11μ thick, coiled tightly to form a three-dimensional helix of up to twenty coils and about $60-200 \times 40-70\mu$ in size. There are normally 6-10 septa to each coil, and the conidial hypha is somewhat constricted at each cross-wall. In all examples observed, the direction of coiling, as seen under the microscope, was clockwise. From observations on a number of isolates, both of *Helicoon* and *Helicodendron*, it seems that the direction of coiling of the conidial filament is a constant feature of many species, and it may be that in the description of helicosporous fungi careful observations of direction of coiling are of considerable taxonomic importance. The spores proliferate fairly readily to form small clusters, proliferation usually occurring from about the middle of the mother spore (Text-fig. 3 and Pl. 9, figs. 2, 5). Young spores are usually hyaline, but after about a week they become brown. They are easily detached and, if a sporing culture is flooded with water, will float to the surface where germination freely occurs, germ tubes arising from several cells of each spore. Those germ tubes originating above the water usually become coiled to form spores, while those immersed in the water give rise to a branched septate mycelium (Text-fig. 4).



Text-fig. 1. *Helicodendron giganteum* n.sp. Spores at various stages of development (from pure culture).



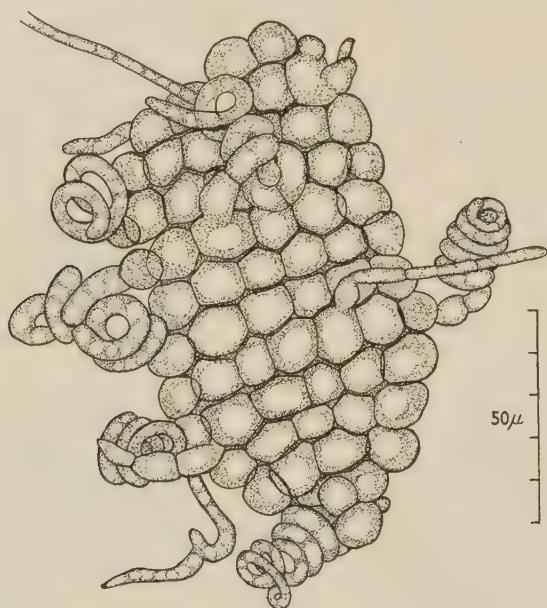
Text-fig. 2.

Text-fig. 3.

Text-fig. 2. *Helicodendron giganteum* n.sp. A fairly mature spore (from pure culture).

Text-fig. 3. *Helicodendron giganteum* n.sp. A group of spores as they appear on the surface of a pure culture on malt agar.

Isolations in pure culture on 2 % malt agar were easily obtained from spore suspensions. The colour of the colonies obtained was somewhat variable, some becoming greenish-black, some pink, and some colourless. After a few days' exposure to light spores developed on the surface of all three types, but sporulation was most profuse on the coloured colonies. Subcultures from colonies with no pigment gave rise to non-sporing colonies. One subculture from a pink colony developed dark freely sporing sectors and colourless non-sporing sectors. Subsequent subcultures from dark colonies and sectors gave rise to cultures with rather less pigment, but spore production remained good (Pl. 9, fig. 3). The rate of growth of the fungus on malt agar was much the same for all types of colony, a 5-day-old culture grown at 20° C. having a diameter of about 6 mm.

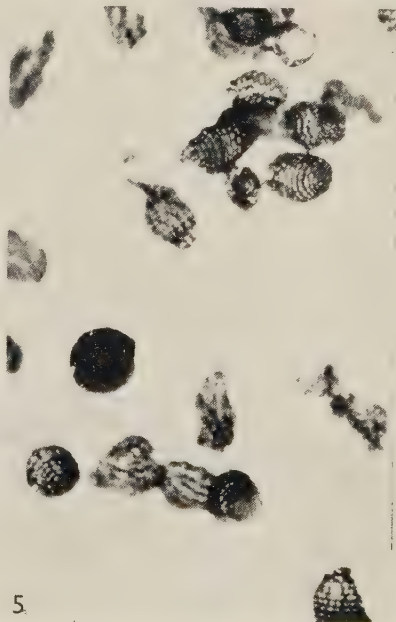
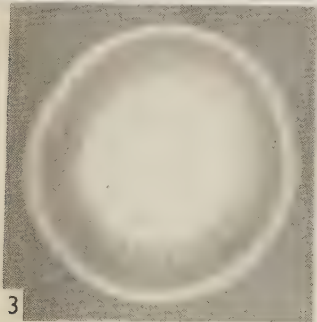
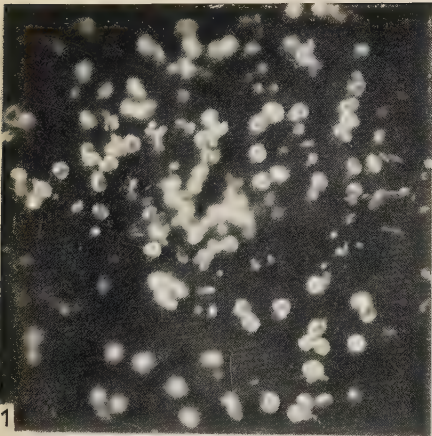


Text-fig. 4. *Helicodendron giganteum* n.sp. A germinating spore from pure culture, after 2 days in 2 % malt broth.

The fungus also grows well on submerged sterilized beech leaves in sterile water, mycelium being produced not only in the leaves but also throughout the medium. If, some weeks after inoculation, such leaves are placed in sterile Petri dishes under damp illuminated conditions, spores are produced freely on the surface of the leaf (Pl. 9, fig. 4).

At a later stage it is hoped to discuss the physiology of these helicosporous fungi. It may be of interest to note that some species at least (*Helicodendron triglitzensis* and *H. tubulosum*) can be grown on malt agar in an atmosphere of nitrogen and sporulate freely. It seems, therefore, that it is the presence of a gas phase rather than the necessity for air that determines sporulation.

The genus *Helicodendron* (type species *H. paradoxum* Peyronel, 1918) is characterized by a spore coiled more or less tightly to form a three-



dimensional helix which proliferates to form daughter coils. This genus differs from the somewhat similar *Helicoon* in the proliferation of the spores. The fungus described in this paper is clearly a species of *Helicodendron*, but differs from all others in the larger size of its spores. The nearest known species is *H. tubulosum* (Riess) Linder, which I have studied not only from my own isolates but also from one in the Baarn collection. Certain of my isolates of *H. tubulosum*, in which coiling is clockwise, appear to approach more closely to *H. giganteum* than do others, including the one from Baarn, where coiling is anti-clockwise. Young spores of both *H. giganteum* and certain isolates of *H. tubulosum* are very much alike both in size and form but enlargement is much more rapid in *H. giganteum* where the fully developed spore is often 3-4 times as long as in *H. tubulosum*. In all species of *Helicoon* and *Helicodendron* that I have examined the young spores are small, and enlargement, by apical growth of the conidial filament and by swelling of the individual cells, is relatively slow. It is not easy to define the lower limit of spore size because a spore that is small because it is immature cannot easily be distinguished from one that is both small and mature.

My thanks are due to Dr H. M. Canter (Mrs J. Lund) who collected the material from which this fungus was isolated, to Miss A. L. van Beverwijk at Baarn for her isolate of *Helicodendron tubulosum*, and especially to Professor C. T. Ingold under whose direction this investigation is being carried out.

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EXPLANATION OF PLATE 9

Helicodendron giganteum n.sp.

- Fig. 1. Spores on the surface of a beech leaf collected from Windermere. (Photographed by reflected light.) $\times 35$.
Fig. 2. Spores at all stages of development from pure culture on malt agar. $\times 80$.
Fig. 3. Pure-sporing culture of fungus on malt agar, grown at about 18-20° C. for 1 month. Natural size.
Fig. 4. Pure sporing culture on a beech leaf. Natural size.
Fig. 5. Spores at all stages of development produced on the surface of the water from a pure culture on beech leaves submerged in shallow water in a Petri dish. $\times 100$.

(Accepted for publication 19 October 1950)

ZALEWSKI'S '*CLATHROSPHAERA SPIRIFERA*'

By AGATHE L. VAN BEVERWIJK

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(With Plates 10-12 and 10 Text-figures)

By observations upon pure cultures of clathroid spores it has been proved that Zalewski based his description of *Clathrosphaera spirifera* upon a mixture of four different fungi. A redescription of genus and species is given. The name *Clathrosphaerina zalewskii* is proposed. The term aero-aquatic fungi is suggested to denote those fungi that grow under water but produce spores above the water-level.

In 1888 Zalewski described a fungus which he named *Clathrosphaera spirifera*. In the life history of this fungus Zalewski observed four different spore forms:

(1) The clathroid spore, a spore in the shape of a hollow sphere with a perforated wall, a spherical network, hence the generic name of the fungus (*clathri* = trellis) (Pl. 10, fig. 1).

(2) The helical spore, a spiral spore, coiled like a corkscrew, hence the specific name.

(3) A sclerotium-like spore, to which Zalewski gave the name of 'spore-ball'.

(4) Cylindrical spores.

Chlamydospores were also observed in the mycelium of the fungus. In text-books of mycology we find this fungus mentioned with some doubt as to its true characteristics and systematic position. Lindau, in Engler and Prantl's *Die natürlichen Pflanzenfamilien*, Teil 1, Abt. 1**, p. 452, 1900, classifies *Clathrosphaera* with the Mucedinaceae, Helicosporae, with the remark:

Leider ist die Abhandlung nur Polnisch geschrieben, und die hier abgedruckte Beschreibung ist nach den Figuren entworfen. Eine Gewähr für die absolute Richtigkeit derselben, sowie für die Stellung des Pilzes an dieser Stelle kann ich daher nicht übernehmen.

In Rabenhorst's *Kryptogamenflora*, Bd 1, Abt. ix, pp. 277-279, 1910, Lindau gives the following description of the genus and species:

Clathrosphaera Zalewski

Hyphen verzweigt, kriechend, septiert. Konidien an kleinen seitenständigen Aestchen entstehend, einzeln gebildet, sprungfederartig aufgerollt, dunkel gefärbt, unseptiert. An gleichen Stellen auch Kugeln entstehend, welche hohl und gitterförmig durchbrochen sind, dunkel gefärbt.

Die Gattung gehörte, wie ich mich an Reukauf'schen Exemplaren überzeugen konnte, zu den Dematiaceen, nicht zu den Mucedinaceen, wohin ich sie zuerst gestellt hatte.

Clathrosphaera spirifera Zalewski

Mycel kriechend, fast hyalin, gar nicht oder nur sehr spärlich septiert, verzweigt, häufig anastomosierend, Konidien an seitlichen kurzen Aestchen sitzend, oft ganze Aestchen dicht besetzend, schneckenförmig aufgerollt, mit bis sieben Windungen,

rauchfarben, septiert. Gitterkugeln am Ende von oft vielfach septierten Aesten gebildet, dunkelfarbig, 25–35 μ im Durchm., durch Zweigbildung mit nachfolgender Anastomosierung an den Enden der Trägerstiele einzeln stehend.

Whether Reukauf's specimen of this fungus, as it was observed by Lindau, showed the clathroid spores as well as the helicoid spores is not quite clear. From the fact that Lindau ranges it under Dematiaceae, I wonder if he has really seen either *Clathrosphaera* or the helical spores which Zalewski attributes to his fungus. According to Zalewski's description the networks as well as the helical spores are hyaline. There also seems to be uncertainty as to the septation of the helical spores. In the description of the genus, Lindau describes them as non-septate, whereas in the species diagnosis he describes the helical spores as septate.

A fungus with helical spores of exactly the same shape and size as those which Zalewski attributed to *Cl. spirifera* had been described before by Riess (1853) under the name *Helicomycetes tubulosus*, syn. *Helicoon tubulosum* (Riess) Sacc., *Helicodendron tubulosum* (Riess) Linder. This same fungus with helical spores was also collected and cultivated by Linder (1929). In pure cultures of this strain none of the other spore forms of Zalewski's fungus ever occurred. Linder, who studied a full translation of Zalewski's paper, concludes his description of *Helicodendron tubulosum* with the words: 'There must remain some uncertainty as to the true life history of *Clathrosphaera* until it is again collected and the life history studied in pure culture.'

From the above-cited descriptions as well as from Linder's remark, it is clear that some problems concerning *Clathrosphaera spirifera* are still to be solved. As Zalewski's publication is written in Polish and is therefore little accessible, a short survey of his article will be given first.*

Zalewski had collected some decaying leaves and moss from an old well near Châtenois (Alsace, France, June 1883). In his laboratory at Strasbourg after 10–12 days he observed a fine white powder on the surface of an elm leaf which was partly sticking out of the water. When seen under the microscope the powder proved to consist of fungus spores of a very peculiar shape. 'The organism has the shape of a sphere composed of a network with six-sided mazes, empty inside—that is to say—filled with air' (Pl. 10, fig. 1).

De Bary, to whom Zalewski showed the organism, encouraged him to continue the study of his fungus. Culture methods were still primitive in those days. Zalewski grew his mould in 'moist chambers' on slides, in sterilized water with some dextrose, or in drops of the water in which he had found the fungus. Contaminations were frequent. Only one of his cultures, grown from four networks, remained pure. This culture was taken to Poland, where Zalewski continued the study of the organism. 'I put the mould culture in a glass jar with the decaying leaves on which it began to grow; on my way, travelling about 200 miles, I carried the glass in my hand nearly all the time.' So his four-spore pure culture was transported in the same glass as the decaying leaves!

When back in Crakow, Zalewski resumed the study of his mould at the laboratory of Prof. Rostafinski. He there cultivated the fungus on different media: slices of carrot and potato, decoctions of carrots, of horse dung, and again in solutions of lactose or dextrose, the concentrations of which are not mentioned. 'I then noticed important changes on the above-mentioned leaf and on the surface of the water in the glass jar. The spherical networks are less numerous, and a great number of spirally coiled hyphae, consisting of three to seven, or even ten coils, closely touching each other, have appeared.' Zalewski grew these spirals and networks on pieces of the original leaf 'with not too many other organisms', and on several other media. From observations on these cultures he con-

* (Mr Lubienski of the linguistic department of Amsterdam University kindly translated Zalewski's article into Dutch for me.)

cluded that 'the spherical networks as well as the spirals when reproducing, only bring forth their own shape, yet the connection between both groups could be ascertained without any doubt'.

Frequently his cultures were contaminated with Infusoria, Penicillia and other fungi; only some of them remained 'pure' throughout the duration of his investigation, i.e. from October 1883 till July 1884, when, unfortunately, the living material of the mould got lost. In December 1883, when the temperature in his working room was very low, on the leaf which he still kept in the same glass jar Zalewski observed 'spherical bodies, colourless and yellowish, much larger than the networks'. The surface of these 'spore balls' was composed of very many spherical cells. They measured 66–83 μ . In a moist-chamber culture the surface cells touching the solution germinated, a mycelium developed and on short aerial hyphae some new spherical bodies were formed by repeated branching of end-cells. Only very rarely young networks were formed in these cultures. 'On the whole, cultures of the spherical bodies do not produce either networks or spirals.' Older spherical bodies were hollow inside, no asci were ever observed. The fact that every cell of this 'spore ball' could germinate even after a period of drying out, also supports the supposition that these bodies had no relation to the perithecia of Penicillia, although they resembled these organisms in shape. Zalewski then compared the different organs of his fungus to similar structures of other fungi.

Spores in the shape of spherical networks are unknown in the realm of the fungi. On the other hand, spiral structures are found in other fungi, e.g. in *Ctenomyces serratus* Eidam. Rees described corkscrew-like structures in the development of *Coprinus stercorarius*, not unlike a young carpoonium of *Eurotium*. The spirals of his mould, however, Zalewski said, were different as they had a more regular barrel-like shape with 5–9 coils closely touching each other, each of these cells being able to germinate.

The 'spore balls' allow a more direct comparison with other fungi. Eidam, in his researches on the development of Ascomycetes, described similar structures in *Helicosporangium parasiticum* and *Papulaspora aspergilliformis*. Woronin observed organs which resemble the 'spore balls' in *Tubercinia trientalis* Berk. & Broom (Ustilagineae). Although Zalewski did not work out in detail this comparison, he suggested that the genus *Clathrosphaera* should be best placed 'in the gap that exists between the Pyrenomycetes and the Ustilagineae'.

To conclude his article Zalewski resumed the life cycle of *Cl. spirifera*. 'The networks mainly develop in spring and summer. During the latter part of the summer, the spirals get more numerous, they thrive until late in the autumn, then, about November, the "spore balls" develop and can be observed until April. These data were obtained from observations upon the mould growing in the glass jar and in the water from the well in which I discovered the mould. In cultures in moist chambers the same organs developed in about the same periods and number. In summer the spore balls were not seen any more; they have to be considered as winter organs.'

The spirals, networks and also the spore balls are filled with air and must be regarded as organs to keep the fungus floating on the water. Zalewski doubted whether they should be considered as conidia, because real conidia were also observed. Chains of cylindrical spores, not unlike those of *Cylindrium candidum* Bonorden, were always present in the culture in the glass jar and in the moist-chamber cultures. These, he pointed out, are the conidia of *Clathrosphaera spirifera*. 'Although they were not seen to develop from spirals or networks, nevertheless they belonged to the same mould, because their hyphae were of the same width and showed the same branching system, quite different from that of other moulds.'

When reading Zalewski's publication, one can but admire his detailed and precise observations upon the development of the different spore forms, which for the greater part could be confirmed by the present study. His conclusions concerning the life history of his fungus, however, were erroneous.

Although Zalewski, when describing the different spore forms, repeatedly states that he did not observe a direct development of one type of spore from the other, it never occurred to him that he might base the descriptions

of the life cycle of his fungus upon a mixture of four different fungi. The fact that they grew on the same decaying leaves, and had about the same type of mycelium, made him think that the different spores belonged to one and the same fungus.

Cylindrical spores are common on decaying leaves in water. Zalewski mentioned that these spores reminded him of those Bonorden described as belonging to *Cylindrium candidum*. Indeed, it seems very probable that the cylindrical spores which Zalewski observed belong to this fungus. However, as he does not give any spore sizes, no definite identification will ever be possible. The helical spores agree very well with the description of *Helicodendron tubulosum* (Riess) Linder, and there seems to be no doubt that the corkscrew spores belong to this species, as already supposed by Linder.

Sclerotium-like spore balls were found several times on decaying leaves in the course of my studies. As I did not succeed in germinating them, no direct comparison with Zalewski's spore balls is possible. They never occurred in pure cultures originating from clathroid spores.

The clathroid spherical bodies certainly are the most conspicuous feature of Zalewski's fungus, and it is curious that they have been so seldom reported since. The fungus is not at all rare, provided one knows where to look for it. It was collected seven times in the course of two years, six times by the writer in the Netherlands and one strain was sent to us by Miss Glen-Bott, who collected the fungus in England (Glen-Bott, 1951). Recently, during the Seventh International Botanical Congress, I collected the fungus in Sweden. Monosporous cultures, started from clathroid spores and cultivated from 1948 up to the present date, never showed any other spore form but the clathroid spores.

A redescription of *Clathrosphaera* based on observations made on pure cultures will be given in the following pages.

MATERIAL

Six strains were collected from three different localities in the Netherlands. Strains no. 1 and 3 grew on decaying birch leaves collected from the surface of a pond at Eerde, the private estate near Ommen, May 1948 (no. 1) and March 1949 (no. 3). Strain no. 2 was collected from a pond in a wood near Baarn. The fungus grew on a beech cupule. A thin web of mycelium was visible between the spines. Spores developed abundantly on the surface of the cupule and along its spines. Strain no. 4 was isolated from a willow leaf, collected from a marshy place near the border of the Kortenhoefse plas (a lake in the province of Utrecht), April 1949. Strain no. 5 grew on a birch catkin, collected from the same locality as strains nos. 1 and 3, June 1949. No. 6 was found on a decaying oak leaf collected from a pond in the surroundings of Baarn, August 1949. No isolations were made. No. 7 was found on *Picea* bark collected from a drainage furrow in a wood not far from Stockholm, Sweden, July 1950. In December 1949 the Centraalbureau voor Schimmelcultures received a culture of *Clathrosphaera spirifera* from Prof. Ingold. This strain was isolated and identified by Miss J. I. Glen-Bott. It grew on a decaying beech leaf, collected from the bottom of a pond, Chislehurst, Kent, November 1948.

From these data it is clear that the fungus can be found throughout the year on various decaying plant materials.

METHODS OF COLLECTING AND CULTURING

Decaying leaves, twigs and other plant material are taken from the surface of water, and taken to the laboratory where the material is put in glass dishes with enough water to keep it damp. The dishes are covered with lids to protect the surface of the leaves from drying out. As the spores of the fungus are readily shed and little mycelium develops on the plant material, the fungus can hardly be detected on freshly collected material. After a few days new spores have been formed and the fungus becomes visible again. The spores are only just discernible by the naked eye as a 'white powder' (Zalewski).

Under a dissecting microscope spores are picked off from the plant material with the aid of a thin glass needle. Different ways of isolating are used. Spores are transferred to Petri dishes with cherry agar, into hanging drops, or micro-cultures are made on slides. The latter methods allow direct observation of the germinating spore. Spores germinate readily by sending out germ tubes from nearly every cell which is in contact with the culture medium (Pl. 11, fig. 2). The young cultures are then transferred to agar slants. Cherry agar proved to be a very suitable medium, both for isolating and culturing the fungus.

Cultural characteristics on different media

(1) On cherry agar in Petri dishes kept at room temperature. Growth variable; even subcultures of the same monosporous isolate may differ considerably in colour and texture. Growth rate rather slow; colonies attaining a diameter of 25–35 mm. in 3 weeks. Colony fluffy* in centre, margin velvety, outer margin consisting of submerged growth with sparse mycelium on the surface. Sectors and zones differing in density of 'pile' often occur. Some cultures are floccose all over their surface. Colonies white to cream-coloured, patches or sectors of yellow (Klincksieck 191–171) may occur, usually near the inoculum. Reverse: maroon brown in centre (Klincksieck 110–114), lighter towards the edge. Spores are formed on the aerial mycelium or in sectors with sparsely developed mycelium on short hyphae erected from submerged mycelial growth. The white powdery patches of spores generally develop near the inoculum, but may stretch to the edge of the colony. Low temperatures are favourable for spore development. Cultures kept in the refrigerator at 4° C. for about the third till sixth week of their development produce spores more abundantly than the controls kept at room temperature. Light does not seem to be of much influence.

(2) Sabouraud maltose agar: Colony cream-coloured, moist, wrinkled, but little aerial mycelium develops. No spores observed.

(3) Malt agar 4 %: Mycelium mat plush to felt-like, creamy white, sometimes light to dark yellow. Spore development poor.

* Terminology used to describe the texture of fungus, as suggested by Mildred K. Nobles (1948, pp. 287–288).

(4) Lutz agar (a decoction of beech wood shavings with some nutritive salts): Fluffy white aerial mycelium, sometimes slightly plumose. Spores on the aerial mycelium.

(5) Carrot plugs: Mycelium plumose, cream-coloured. Spore development poor.

(6) Sterilized lupin stems: Mycelium sparse, a tuft of white fluffy mycelium on top of the stem. Spores mainly develop on the (agar) inoculum and in the aerial mycelium on top of the stem. When kept in the refrigerator for some weeks, the spore development is more abundant and occurs all over the surface of the stem.

(7) Liquid medium: The fungus develops very well in cherry decoction. Glass dishes of 6 cm. diameter are filled with diluted cherry decoction to a depth of about 5 mm. Within a few weeks the fungus spreads from the inoculum all over the dish. Spores develop on short branches of the aerial mycelium. No spores are formed in the liquid medium.

No perfect stage was observed. To ascertain if the fungus was heterothallic, strains of different localities were paired, however, without any result.

Development of the spore

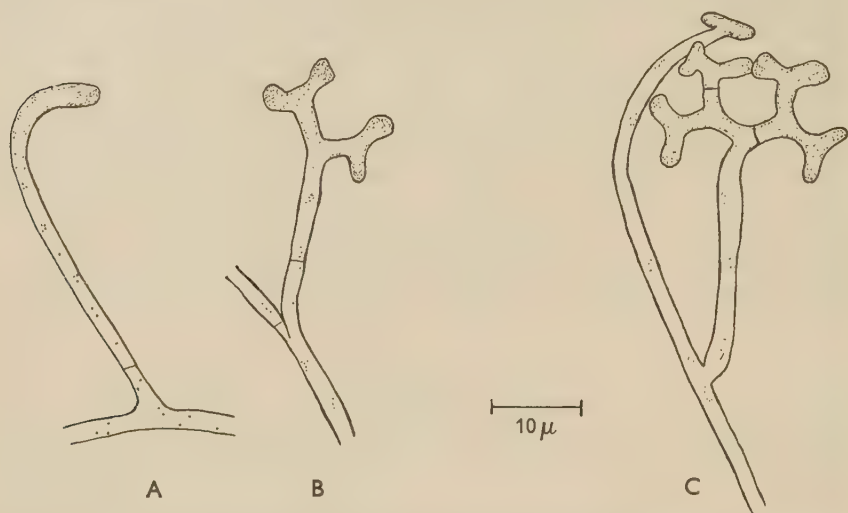
Spores develop on the aerial mycelium, just above the level of the water or culture medium. No spores were observed on submerged hyphae. The conidiophore is a simple side branch of a hypha. It broadens slightly towards its free end (Text-fig. 1 A), then the tip forks and short branches grow out in two directions, forming the bottom walls of the first mesh. The directions of growth of the forking tips are not quite opposite, but the outgrowing branches form an angle of about 120° . The free tips broaden again, fork once more, thus forming the side walls of the first mesh and parts of the walls of four neighbouring meshes (Text-fig. 1 B). Again the free tips fork, when forks of the right and the left of this third set of branches meet, the first mesh of the spore is closed (Text-fig. 1 C, Pl. 11, fig. 1).

As far as I could observe on living and mounted spores (in water or in lactic acid with cotton blue) there is no true anastomosis. The branch tips may grow firmly together at their meeting point, the septum between them, however, is not dissolved. This meeting and growing together of branches of a spore is very rare, if not unique, in the development of spores in the fungi.

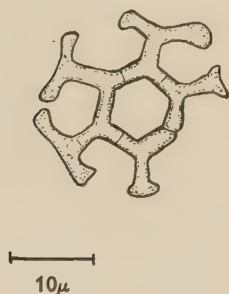
Septa are formed in the walls of the mesh. The septa are often rather indistinct. A regular mesh is six-sided (Text-fig. 2). Meshes as regular as this, however, are rare; five- or four-sided meshes also occur (Text-fig. 3).

One detail in Zalewski's description of the development of the spore I could not confirm. According to Zalewski the walls between the two side branches which have met to close the mesh, disappear, a small bud then grows out from this point. When it reaches the length of a side of a mesh, its free end branches, and so it forms part of the wall of a new mesh again. I have never actually seen this occur. When the two branches met exactly with their tips, no further growth at this point was observed (Text-fig. 4). A new branch may come from another direction, and by touching the meeting point of the first mesh separate two new meshes (Text-fig. 5 A, B).

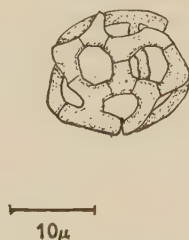
Often the branches do not quite meet with their tips, but a shorter branch touches a longer one, thus the tip of this branch remains free and continues its growth (Text-fig. 6). Soon the branches bend out of the plane of observation because of the spherical growth of the spore. An air bubble is caught in the interior of the developing network. The spore then is no longer transparent, which makes it very difficult, if not impossible, to



Text-fig. 1 A-C. Spore development. A, conidiophore with broadening tip; B, forking tips; C, the first mesh is closed by the meeting of two fork tips.



Text-fig. 2. Detail of spore. A regular six-sided mesh.

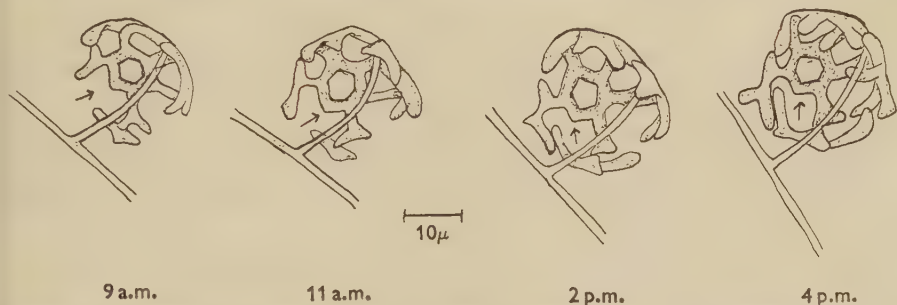


Text-fig. 3. Spore with less regular meshes.

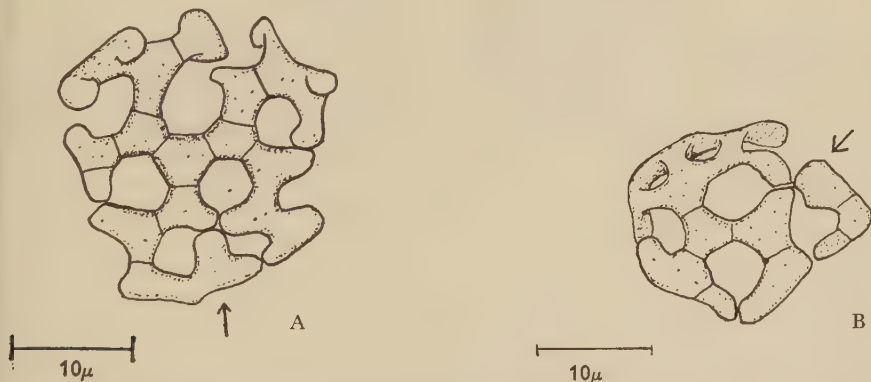
follow the pattern of the growing spore. The growth rate is rather slow (Text-fig. 4). The growth of most spores soon becomes irregular when branch tips fail to meet as they develop at a different rate of growth or not quite in the same plane (Text-fig. 7). A branch tip which remains free continues forking. Thus the spore increases in size by formation of irregular layers of network. The full-grown spore is not hollow. The network also fills the interior of the spore. Occasionally a spore continues growth in one plane for some time, and the development of several meshes can be followed (Text-fig. 8). Some spores are complicated from the very start

because spore development begins not only at the distal end of the conidiophore, but side branches also grow out (Text-fig. 9A, B). The spore sizes are $18-25\mu$ (extremes $15-40\mu$). The filament of the network measures $1.5-3\mu$, the diameter of the meshes it encloses is $4-6\mu$. (Pl. 12, figs. 1, 2 and 3).

The cells constituting the spore are broad triangular to three-armed. Detached fragments of the spore remind one of the pieces of a jigsaw puzzle (Text-fig. 5 A), and the spore itself is not unlike a Chinese ivory cut



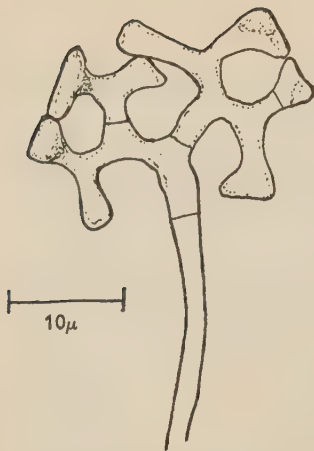
Text-fig. 4. Growth of a living spore on an aerial conidiophore. Culture growing in cherry decoction. At the meeting point of the two tips—indicated by an arrow—no further growth was observed. Under the conditions of the illumination used, cross walls were not visible. The rate of growth is indicated.



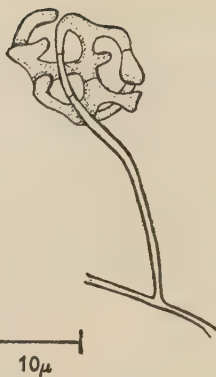
Text-fig. 5A, B. Details of spores. A branch from another direction—indicated by an arrow—touches the meeting point of two fork tips, thus separating two new meshes.

curio. Strange as this spore structure may be, it still answers to the definition of a conidium verum, as it is an asexual spore which at maturity is shed from its conidiophore. Moreover, each cell of this spore can send out a germ tube, where it touches a suitable medium (Pl. 11, fig. 2; Text-fig. 10).

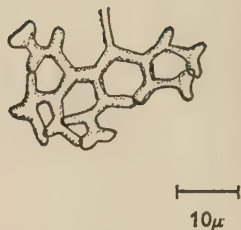
Like many helicosporous Fungi Imperfecti living in this same habitat, this fungus grows partly under water (the mycelium). Partly, however, the fungus lives in the air, as the spores are always produced above the water-level. For this type of fungi the writer would suggest the term 'aero-aquatic fungi'.



Text-fig. 6.



Text-fig. 7.

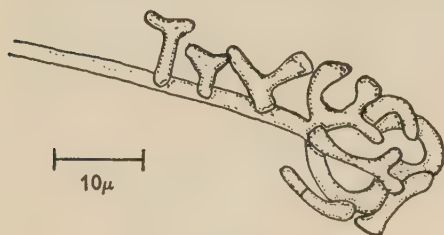


Text-fig. 8.

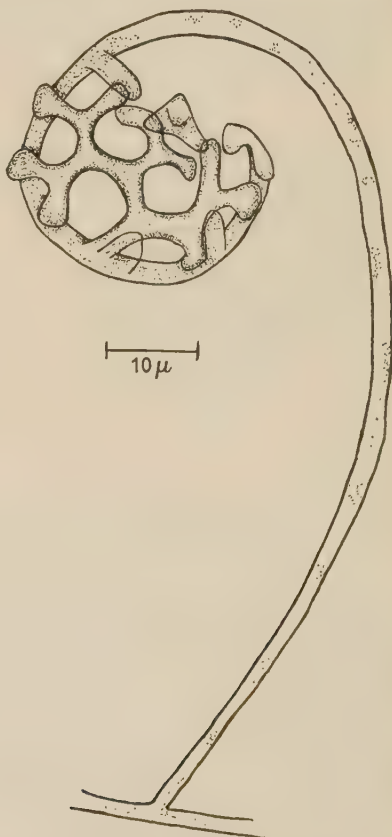
Text-fig. 6. Developing spore. The first mesh is irregular because the fork tips did not exactly meet. The longer branch will continue its growth. Meshes to the left and right are more regular.

Text-fig. 7. Irregular spore development. Branch tips grow out at different rate of growth or not in the same plane, hence they fail to meet.

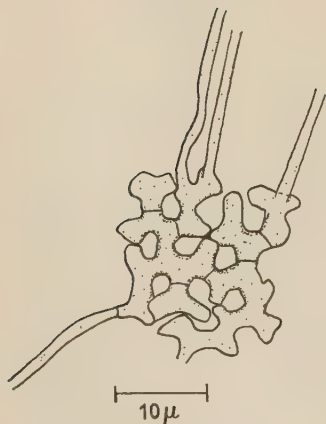
Text-fig. 8. Growth of a living spore. The development continues in one plane for some time, the formation of several more or less regular meshes can be followed. Cross-walls are not visible under the conditions of illumination and magnification used.



Text-fig. 9A.



Text-fig. 9B.



Text-fig. 10.

Text-fig. 9A, B. Spores irregular from their beginning, because side branches from the conidiophore also take part in the spore formation.

Text-fig. 10. Spore fragment in cherry decoction. Several cells are sending out germ tubes.

As it has been proved by observations on pure cultures that Zalewski based his description of the genus *Clathrosphaera* and the species *Cl. spirifera* on a mixture of different fungi, these names are no longer valid. A new genus and species will have to be erected for the above-described fungus. The genus name is very characteristic and should be changed as little as possible; the species name 'spirifera' has no longer any sense, since the spirals do not belong to the fungus. The writer therefore proposes to name this fungus *Clathrosphaerina zalewskii*.

***Clathrosphaerina* gen.nov.**

Fungus aero-aquaticus, mycelio hyalino, repente, septato, ramoso. Conidia hyalina, multicellularia, rete globosum crebra ramificatione convenientibus ramorum extremis partibus effectum.

Aero-aquatic fungus. Mycelium hyaline, branched, septate. Conidium hyaline, multicellular, a spherical network produced by repeated forking and meeting of fork tips.

***Clathrosphaerina zalewskii* sp.nov.**

Fungus aero-aquaticus, mycelio repente, septato, ramoso. Hyphae. $1-2\mu$ crassae. Conidiophora rami simplices mycelii. Conidia supra superficiem aquae exorientia; hyalina, multicellularia, $18-25\mu$ ($15-40\mu$); crebra ramificatione convenientibus ramorum extremis partibus rete globosum efficitur. Maculae retis cinguntur cellulis triangulis aut tribrachialibus, $1.5-3\mu$ latis. Unaquaeque cellula conidii germinare potest.

In membris herbarum sub aqua putrescentibus.

Aero-aquatic fungus. Mycelium hyaline, branched, septate. Hyphae $1-2\mu$ thick. Conidiophore a simple branch of the mycelium. Conidia produced above the water-level. Conidium hyaline, multicellular, $18-25\mu$ ($15-40\mu$), by repeated forking and meeting of fork tips a spherical network is produced. The meshes of the network are surrounded by triangular or three-armed cells, $1.5-3\mu$ broad. Each cell of the spore is capable of developing a germ tube. On decaying plant material in water.

Type material—living and dried cultures of the strain collected from birch leaves, May 1948—will be maintained at the Centraalbureau voor Schimmelcultures at Baarn.

Where to classify this remarkable fungus is a question not easily answered. Zalewski's fungus was ranged with the Helicosporae. Without the helical spores, however, the fungus does not fit any more in this group. The writer would suggest classifying *Clathrosphaerina zalewskii* with the Staurosporae, because a branching structure forms the basic pattern of the spore.

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EXPLANATION OF PLATES 10-12

PLATE 10

Fig. 1. Clathroid spores (after Zalewski, Tab. III, figs. 1, 2).

Fig. 2. Irregular clathroid spores (after Zalewski, Tab. III, figs. 7, 11, 12).

PLATE 11

Fig. 1. Developing spore. The first mesh is closed, neighbouring meshes are being formed. The top of the conidiophore, bent to the left, is broadening to form another spore. $\times 530$.

Fig. 2. Germinating clathroid spores. The spores had been in a drop of cherry decoction for 24 hr.; mounted in lactic acid with cotton blue. $\times 300$.

PLATE 12

Fig. 1. Young spores, still attached to their conidiophores. From a 5 weeks old culture on lupin stem. Mounted in cellulose acetate, dissolved, lactophenol with cotton blue. $\times 420$.

Fig. 2. Developing networks. Culture and mount as in previous figure. $\times 420$.

Fig. 3. Full-grown clathroid spores. Mounted in lactophenol with cotton blue. $\times 1000$.

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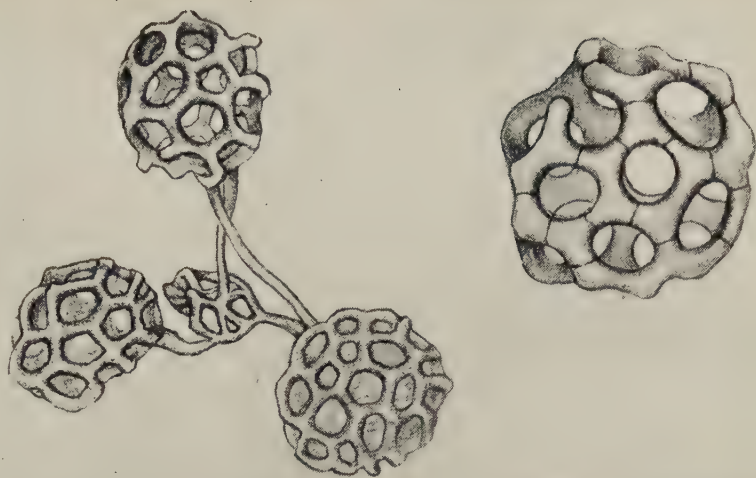


Fig. 1.



Fig. 2.



Fig. 1.



Fig. 2.

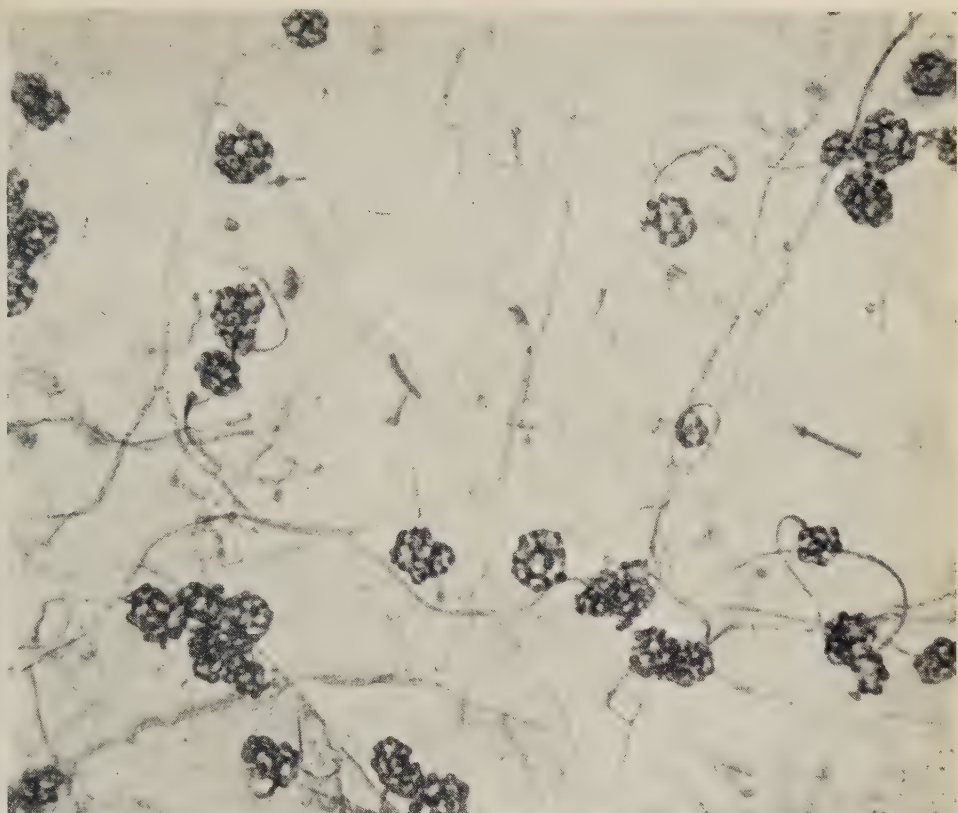


Fig. 1.

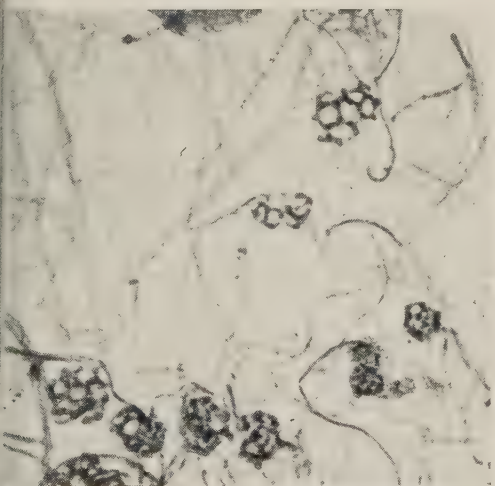


Fig. 2.

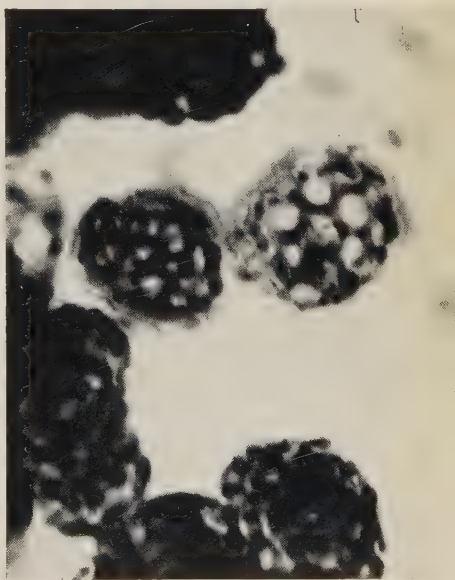


Fig. 3.

A PETRI-DISH MOIST CHAMBER

BY W. G. KEYWORTH

East Malling Research Station

(With Plate 13)

An improved moist chamber, for encouraging fungi to grow from pieces of infected plant tissues, can be made by adding strips of compressed paper pulp to Petri dishes containing the usual moistened filter-paper. The strips absorb sufficient water to keep the filter-paper moist for 3-4 weeks.

In the study of fungus plant pathogens it is often necessary to incubate pieces of plant tissue under moist conditions to encourage fungus growth and sporulation. This practice is especially useful for certain vascular parasites such as *Verticillium* spp. which grow from infected vascular tissue, as it often enables a preliminary diagnosis to be made without isolation of the fungus on agar. I have therefore frequently used it in tracing the extent of invasion of infected plants, etc.

The method usually adopted is to place the pieces of tissue in unsterilized $3\frac{1}{2}$ in. Petri dishes containing a moistened filter-paper. The method has the great disadvantage, however, that the filter-paper usually dries out in a few days, necessitating frequent rewetting and consequent variation in humidity. The subsequent applications of water also tend to soak the plant tissue and suppress fungus sporulation.

The drying of the filter-paper can be avoided if a strip of paper pulp is placed on edge round the wall of the dish and resting on the filter-paper. This strip absorbs a large volume of water and ensures that the filter-paper remains moist for at least 3-4 weeks, which is usually long enough for studies of the nature described. If required for longer periods the pulp strip can be rewetted from a pipette without any free water reaching the plant tissue.

Tests have been made with dishes containing only the moistened pulp strip, but these were found unsuitable for the growth of *Verticillium* from small pieces of tissue because the centres of the dishes remained dry. This method may sometimes be satisfactory, but it appears likely that the continuous percolation of water on to the filter-paper ensures a constantly high humidity.

The strips used were $10\frac{1}{2}$ in. long, $\frac{3}{8}$ in. wide and $\frac{1}{8}$ in. thick when dry and cut from a large 'clarity sheet', i.e. a sheet of compressed paper pulp used in commercial filters. These sheets usually measure 25 in. square and can be obtained from some makers of filter-papers.* The maker will usually cut them into strips for an extra charge, or this can be done on a large guillotine.

* Present price 3s. 10d. per sheet; obtained by the author from J. Barcham Green Ltd, Hayle Mill, Maidstone, Kent.

In making up a moist chamber, the filter-paper and pulp strip are inserted dry and then flooded with 18–20 c.c. of water, all of which is absorbed. The strip swells to twice its original thickness and also increases in length, then just fitting a standard Petri dish. For the study of fungus growth from unsterilized specimens it appears unnecessary to sterilize the dishes or strips, etc., but if they are required for aseptic techniques the chambers can be sterilized in an autoclave and then flooded with sterile water. Sterile chambers have proved of particular value in this laboratory for the germination of seeds under sterile conditions for inoculation experiments. The seeds are sown on the pulp strip or on disks of pulp, and they germinate readily. If the strips are to be used again they can be sterilized in an autoclave and then dried for 24 hr. at 90° C. for storage.

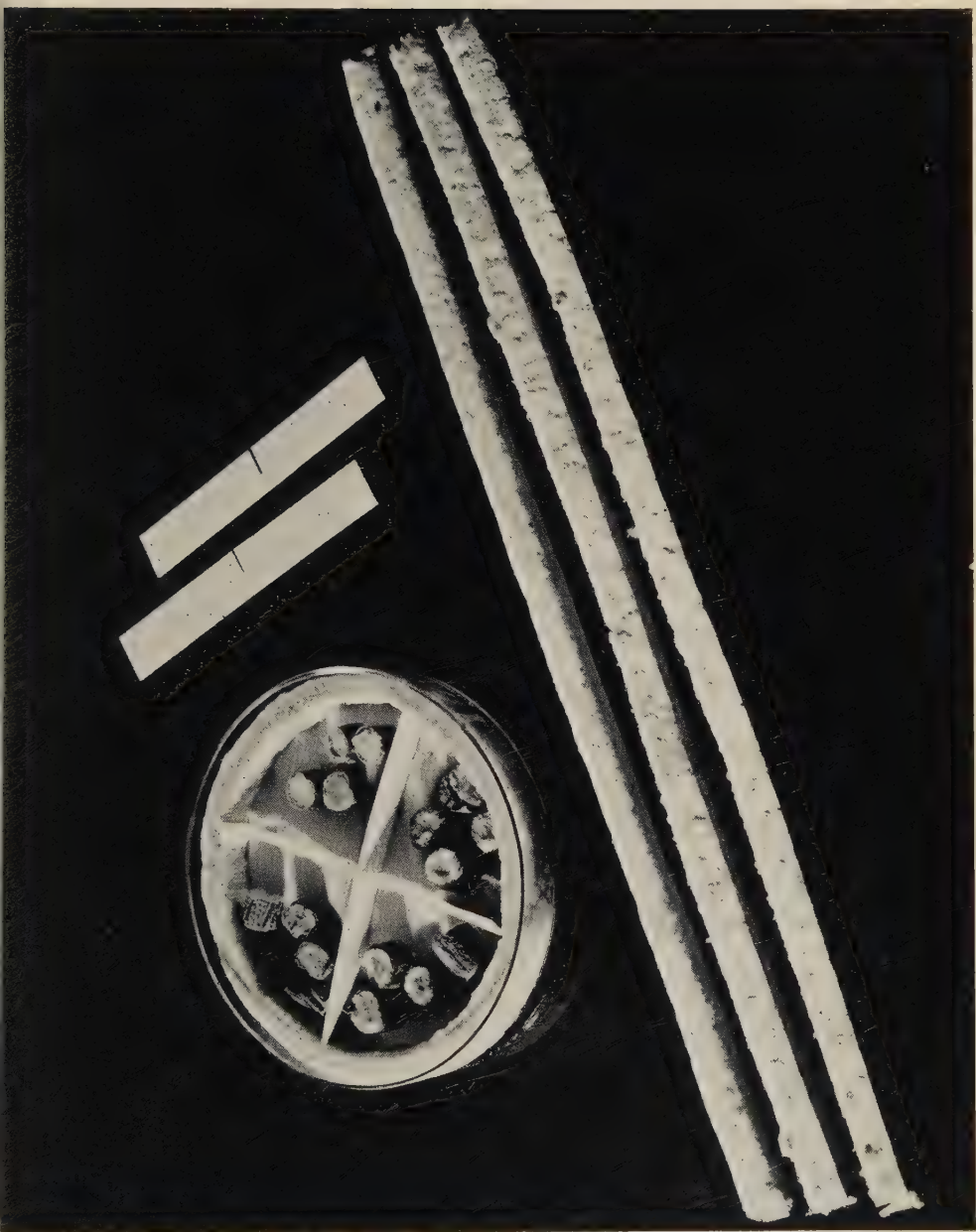
When many specimens have to be examined, a further modification is useful. Each dish is divided into four by means of strips of xylonite measuring $3\frac{1}{8} \times \frac{3}{8}$ in. These are sterilized by immersion in a disinfectant solution.

The materials used are shown in the accompanying illustration.

EXPLANATION OF PLATE 13

A Petri-dish moist chamber and strips of paper pulp and xylonite as used in its preparation.

(Accepted for publication 28 October 1950)



A *PHYTOPHTHORA* CROWN ROT OF *CAMPANULA*

By BETTY J. LEGGE*

Department of Botany, University of Birmingham

(With Plate 14 and 5 Text-figures)

A species of *Phytophthora*, identified as *P. porri*, is shown to be the cause of a hitherto undescribed crown rot of *Campanula*, the symptoms of which are described. The fungus failed to infect leeks under the conditions of the experiments carried out. Sporangia were found in soil round diseased plants, and the life of *Phytophthora* in the soil is discussed in relation to this discovery.

This disease affected a variety of *Campanula persicifolia* L. grown for show purposes by Mr Holding of Evesham. The only other recorded attack of *Phytophthora* on *Campanula* is that of Tucker and Benson (1934), who reported a black decay of the stems, leaf bases and peduncles of *C. persicifolia* caused by an unidentified species.

SYMPTOMS

Infection of the *Campanula* becomes apparent at any time between April and July, and no plants once affected have been seen to recover. Typical symptoms of the disease are shown in Pl. 14, fig. 1 B and in Text-fig. 1. In plants obviously diseased the crown region is grey-brown to chocolate-brown; this discoloration spreads upwards into the leaf bases and flowering shoots and downwards into the roots. The upper parts of the roots are pale pink to brown and later turn dark brown and disintegrate, freeing the leafy rosette from the roots.

Mycelium of a species of *Phytophthora* was present in the newly invaded tissues of affected plants, and oogonia, antheridia and oospores were found in all diseased parts. The range in diameter of 150 oogonia and oospores was 30–42 and 24–32 μ respectively. Sporangia were not observed unless damp conditions prevailed, when a few protruded from the region of the crown and leaf base. They were obtained in profusion, however, on immersing infected material in water. The range of 107 sporangia was 33–60 $\times$ 22–35 μ .

ISOLATION

Associated organisms rendered isolation of the *Phytophthora* difficult, but a standard technique was eventually devised. Leaf bases were washed in sterile water, surface sterilized in a 0.35 % solution of hypochloride of lime for 5 min., or in a 0.1 % solution of mercuric chloride for 4 min., and washed again. Small portions were excised at the junction of healthy and diseased tissue, and, after a final washing, were dried on sterile filter-paper and pushed into oat-extract agar.

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Text-fig. 1. Plants of the *Campanula* variety exhibiting crown rot. A, flowering shoot showing decorticated base; B, flowering shoot; C, daughter plant removed from the crown region of an affected plant; D, plant at the leafy, rosette stage showing severance of the roots. (Discoloured regions stippled.)

Over 300 attempts at isolation were made and fourteen cultures of the *Phytophthora* were eventually obtained. Numerous cultures of *Pythium intermedium* de Bary and *Rhizoctonia solani* Kühn (*Corticium solani* (Prill. & Delacr.) Bourd. & Galz.) and two cultures of a *Pythium*, referable to *Pythium violae* Chesters & Hickman, were isolated and considered as possible pathogens of the *Campanula*.

PATHOGENICITY EXPERIMENTS

As preliminary experiments indicated that the *Pythium* and *Rhizoctonia* species were not causal organisms of the crown rot, only experiments involving the *Phytophthora* are described.

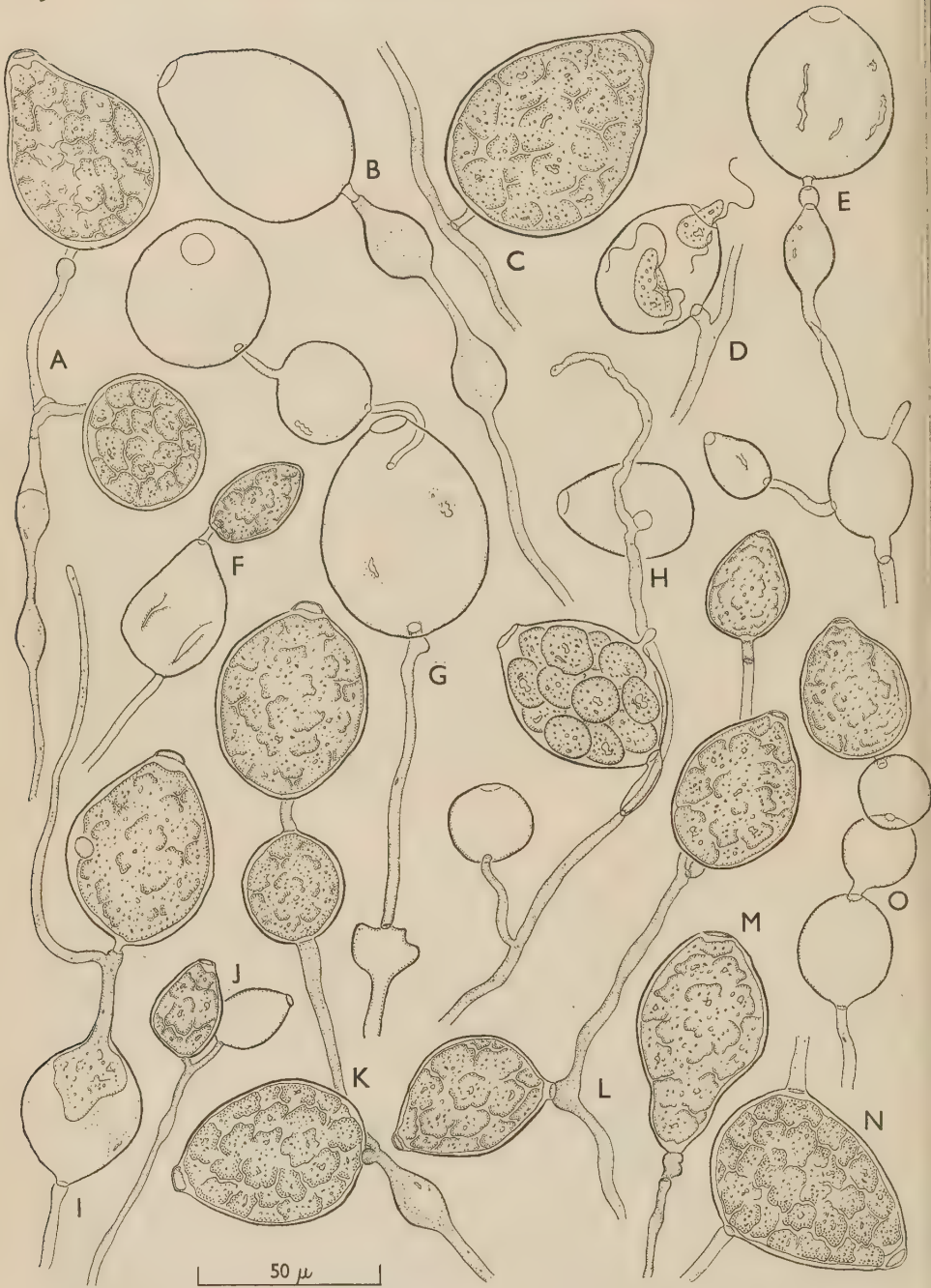
Healthy plants of the *Campanula* variety naturally infected were not available in quantity and *C. persicifolia* was employed throughout the experiments. This species became infected in soil which nine months previously had borne plants of the *Campanula* variety with crown rot. Representative isolates of the *Phytophthora* grown on sterile *Campanula* leaves were shown to be capable of killing *Campanula* through wounds made in the leaf bases. These isolates did not cause infection when a suspension of the mycelium in water, obtained by scraping the surface of an oat-agar culture, was sprayed on the inner sides of unwounded leaf bases. Plants growing in non-sterile, limed or unlimed compost and garden soil, developed the typical symptoms of crown rot two weeks after suspensions of the mycelium had been sprayed on the surface of the soil around the plants (Pl. 14, fig. 2). Infection was also obtained when the upper roots were exposed and similarly inoculated. The presence of the *Phytophthora* in diseased specimens was always demonstrated. Control plants in these experiments all remained healthy, sterile *Campanula* leaves or sterile water being employed as inocula.

DESCRIPTION OF THE PHYTOPHTHORA

This grows profusely, but slowly, on agar media. Its optimum growth temperature is about 20° C.; at 27° C. growth is completely retarded but the fungus remains alive. Aerial hyphae are produced on media with high nutrient content. Surface and submerged hyphae are meandering, 2–8 μ in diameter and aseptate when young, with branches usually constricted at the point of attachment. The hyphae sometimes coil and later become septate, the finely granular, refringent protoplasm receding to certain portions delimited by septa.

Chlamydospores as defined by Blackwell (1949) are absent, but intercalary, spherical or irregular, hyphal swellings occur singly, or typically in chains, in solid or liquid media.

Sporangia (Text-fig. 2) are produced within 24 hr. on organic substrata immersed in water or Petri's solution (1917). They vary in shape, but are typically ovoid to obpyriform and may be terminal, lateral or intercalary. True pedicels, about 5 μ long, are sometimes present on detached, mature sporangia. Papillae are usually conspicuous and shallow, occasionally inconspicuous. Swarmspores are released directly or into an evanescent vesicle, while undischarged sporangia may renew growth apically to form



Text-fig. 2. A-O, sporangia of the *Phytophthora* from one-week-old cultures on sterile *Campanula* leaves in aerated Petri's solution. D, a sporangium with two swarmspores trapped so that they could be drawn partly by camera lucida, partly free-hand, and H, two empty sporangia and one sporangium with swarmspores which have rounded off.

secondary sporangia. Sporangiphores may be laxly branched, with growth resumed racemously or cymosely, but never by proliferation through a previous sporangium.

Oogonia, antheridia and oospores are produced within two weeks in oat and maize agars (Text-fig. 3). Oogonia are globose to pyriform and yellow to light brown with a smooth, thin wall. Antheridia are variable in shape, occasionally amphigynous but usually paragynous, and one or two are associated with each oogonium. Oospores are spherical to subspherical and yellow to light brown with a smooth wall about 3μ thick.

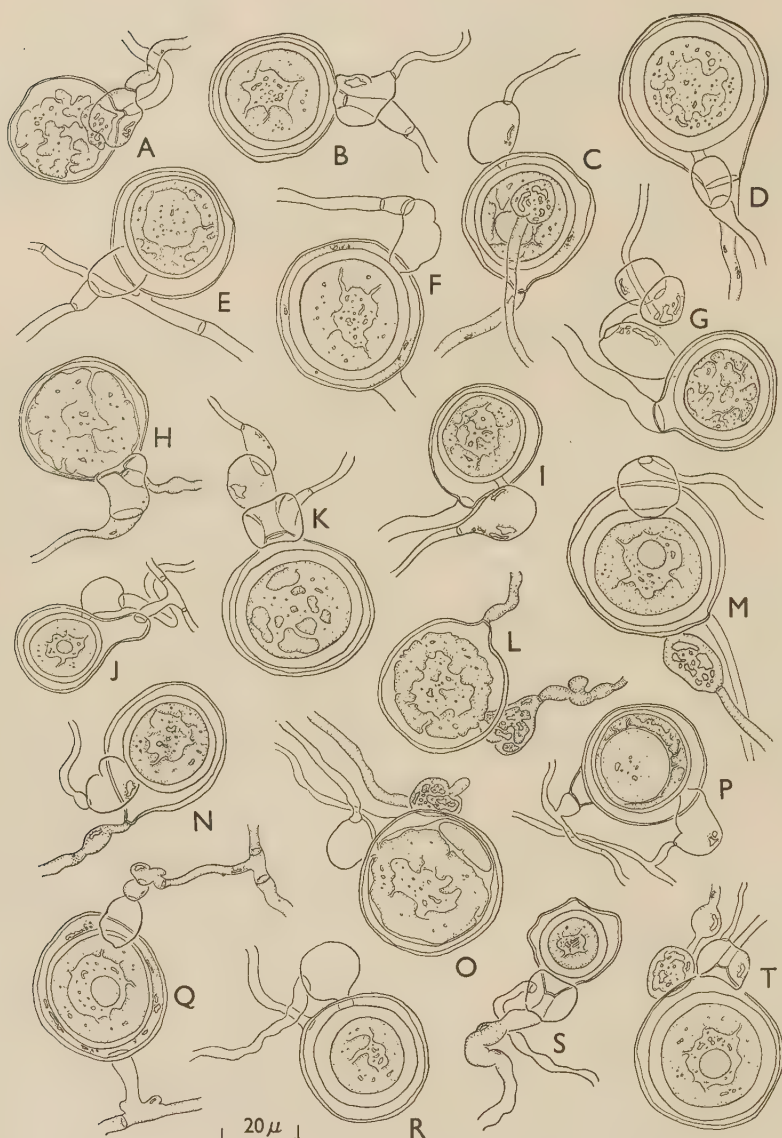
IDENTIFICATION

Using Leonian's key for the identification of *Phytophthora* species (1934), the *Phytophthora* from *Campanula* can be assigned to *Phytophthora porri* Foister, isolated from leeks (1930), on account of its growth on malt agar, failure to produce sexual organs on transference from pea broth to distilled water, and lack of growth but ability to live at 27°C . Using Tucker's monograph of the genus (1931), the fungus can be referred to *P. syringae* Kleb. on account of its growth on malt agar, predominantly paragynous antheridia, lack of growth at 27°C . and pathogenicity to wounded apple fruits; this author apparently did not have a culture of *P. porri*.

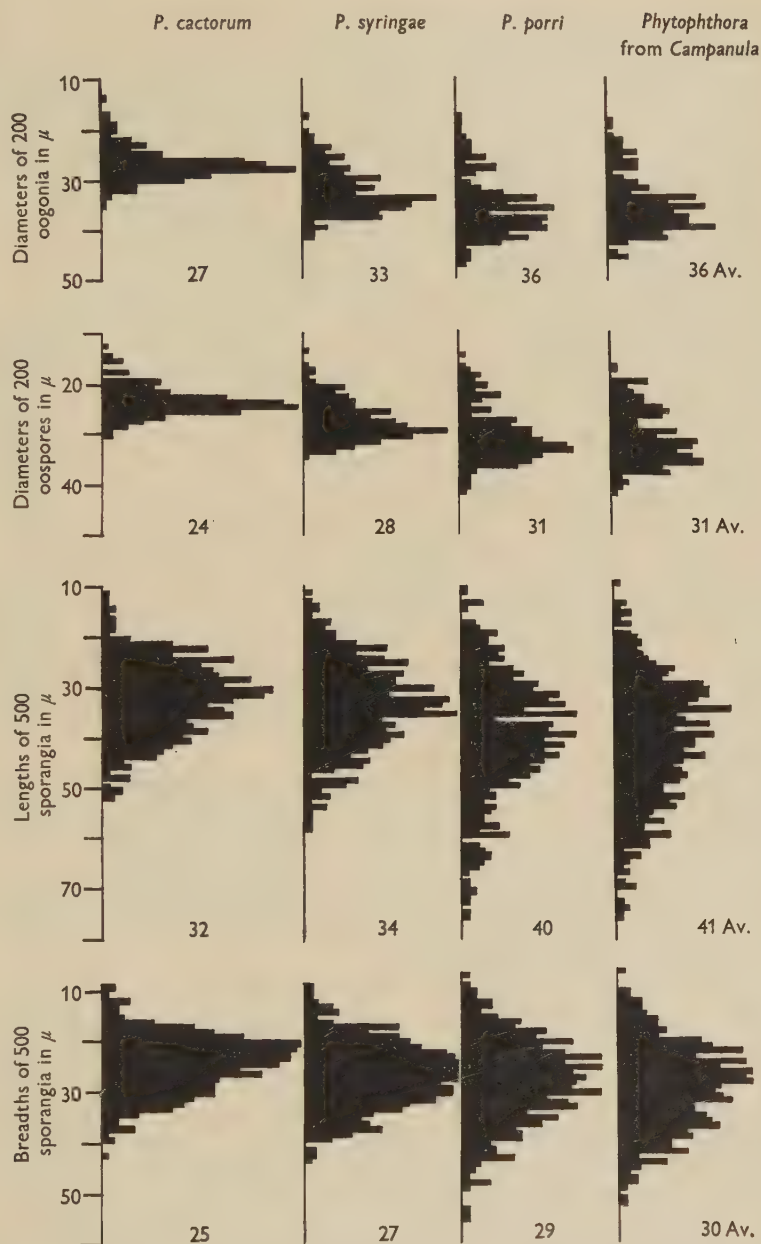
My *Phytophthora* can certainly be included in the group containing *P. cactorum* (Leb. & Cohn) Schroet., *P. syringae* and *P. porri*, and I compared and contrasted it with cultures of these species. Miss E. Blackwell, formerly of Royal Holloway College, London, kindly provided a culture of *P. cactorum*, and Dr C. J. Hickman a culture of de Bruyn's isolate of *P. syringae*, to both of whom my thanks are due. I isolated *P. porri* in 1942 from leeks affected with White Tip, and the identity of the fungus was kindly confirmed by Dr Foister. Numerous measurements of the reproductive organs of these species were made by the writer and histograms of these are shown in Text-fig. 4. The ranges, mode ranges and averages of *P. porri* and the *Phytophthora* from *Campanula* are noticeably similar and contrast with those of the other species. Further morphological examination endorses this resemblance between the two fungi.

Pathogenicity experiments were conducted with all four species of *Phytophthora* under discussion. *Campanula persicifolia* and *Allium porrum* L. were wound-inoculated and also exposed to infection from suspensions of mycelium on the soil around the plants. *Phytophthora cactorum* and *P. syringae* failed to infect either host. The *Phytophthora* from *Campanula* caused the usual crown rot in *Campanula* both through wounds and from the soil, while *Phytophthora porri* produced small, localized lesions in wound-inoculated leek leaves; these occasionally resulted in the death of the whole leaf, but the isolated symptoms of White Tip disease never spread into the rest of the plant. The leeks were not infected by spraying the soil around the plants. *Campanula* was not infected by *Phytophthora porri*, and the leeks were not infected by the *Phytophthora* from *Campanula*.

The conclusion reached is that the *Phytophthora* from *Campanula* is *Phytophthora porri*, which, however, proved non-pathogenic to leeks under the conditions of my experiments.



Text-fig. 3. Sexual organs of the *Phytophthora* from cultures up to eight weeks old on sterile *Campanula* leaves, C, E, F, J, M and P; oat agar A, B, G, H, K, L, Q, S and T; and oat-extract agar D, I, N and R. D, oogonium, oospore and one paragynous antheridium; K, oogonium, oospore and one amphigynous antheridium; M, oogonium, oospore and two paragynous antheridia.



Text-fig. 4. Histograms based on measurements of sporangia, oogonia and oospores of certain species of *Phytophthora*. Sporangia were measured from cultures up to two weeks old on sterile oat agar, *Campanula* and leek leaves in aerated Petri's solution. Oogonia and oospores were measured from cultures up to eight weeks old on oat and maize agars. (The average is shown under each histogram.)

THE LIFE OF *PHYTOPHTHORA* IN THE SOIL

In *Phytophthora* infections, soil in which plants have become diseased has been demonstrated to be capable of leading to re-infection after a lapse of time. De Bruyn (1922) presented evidence of this compiled from many authors, and she explained it by the saprophytism of the fungus in the soil. Dufrenoy (1926) also pointed out that saprophytism in the soil was to be expected of species of *Phytophthora* pathogenic to susceptible hosts.

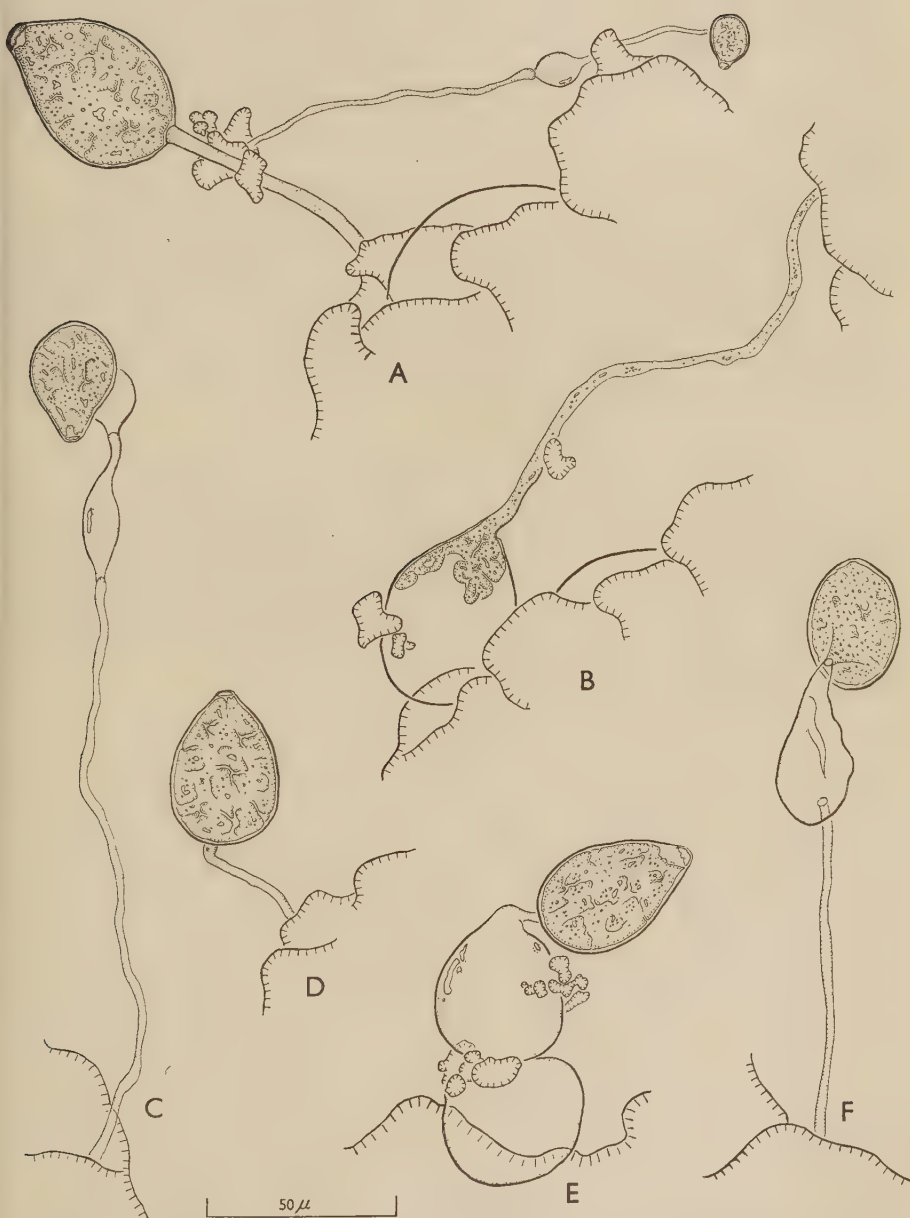
De Bruyn successfully cultured certain species of *Phytophthora* for periods of over twelve months on sterile bog soil, leaf mould, clay and sand, in which they produced mycelium, sexual and asexual organs. She also inoculated non-sterile soil, but was unable to demonstrate visually the presence of the *Phytophthora* except by successful pathogenicity experiments performed six months later with the host plants. Foister grew *P. porri* in sterile soil and found oospores and abundant sporangia in that medium. The *Phytophthora* from *Campanula* has also been grown on a sterilized mixture of soil, sand and macerated *Campanula* leaves, with the formation of mycelium, sexual and asexual organs after four weeks.

Growth on sterile media cannot be reliably compared with growth in nature. It was for this reason that non-sterile composts and soil were employed in the pathogenicity experiments. The success of the inoculations from soil indicates the ability of the *Phytophthora* to withstand the biological antagonism of the soil fauna and flora. The inoculum of a suspension of mycelium in water was intended to disturb as little as possible the life of the soil micro-organisms already present, for Garrett (1934) recorded that the addition of organic media to the soil did not favour infection because of increased micro-biological activity.

Soil from the crown region of diseased *Campanula* plants was studied in modified Cholodny (1936) soil chambers. A very small quantity of soil was spread over the centre of a cover-glass. This was inverted over a van Tieghem cell one-third filled with water, and was fixed to the upper surface of the cell by Vaseline. The apparatus could be transferred easily to a microscope stage, and there the growth of organisms from the soil observed and drawn under high magnification.

A dozen recognizable sporangia of a *Phytophthora* developed in soil chambers at various times, and drawings of some of these are shown in Text-fig. 5. The sporangia were presumed to be those of the *Phytophthora* causing the crown rot, as the soil producing them had come from round diseased plants. No swarmspores were released, but this was thought to be due to lack of aeration—the fault of this technique. The sporangia and any hyphae produced from them were eventually destroyed by bacteria which are stimulated to abnormal development by this method of culture.

Waterhouse (1930) noted that many species of *Phytophthora* form sporangia only in inorganic liquids, or more readily there than on solid media. Petri (1917) devised a mineral solution which would induce sporangial formation in species where few, or none, had previously been obtained. Mehrlich (1934) used a non-sterile soil leachate for the same purpose. Agar inocula of *P. syringae*, *P. cactorum*, *P. porri*, *P. cryptogea* Pethyb. & Laff. and the *Phytophthora* from *Campanula* all produced numerous sporangia in sterile,



Text-fig. 5. Sporangia from soil around the crown region of diseased *Campanula*, produced after ten to sixteen days in soil chambers. B, shows the sporangium of A germinating to produce a long hypha which meandered between the soil particles for some days. (The outline of the soil particles is indicated by open shading.)

waterlogged soil. The sporangia were found 2–3 cm. away from the inocula and readily released swarmspores when the cultures were aerated. Smith and Smith (1906) found that sporangia of *Phytophthora citrophthora* Fawcett were rare in culture, but were produced abundantly and typically in moist soil in contact with affected citrus fruits. Hickman (1940) also stated that sporangia of *P. fragariae* Hickman develop naturally on the root surface of diseased strawberry plants in moist soil.

These observations are significant in relation to the incidence of soil-borne *Phytophthora* diseases. The discovery of sporangia at soil level on infected *Campanula* suggests that the fungus spreads into the soil thus facilitating infection of neighbouring plants. Furthermore, the actual formation of sporangia in the soil in the absence of the host makes it highly probable that this species of *Phytophthora* is capable not only of independent existence in the soil, but also of asexual reproduction in that medium. It is, therefore, suggested that a normal mode of infection is from swarmspores from sporangia on mycelium in the soil.

I am extremely grateful to Mr Holding for his kindness in giving me access to his garden to study this disease at first hand, and for diseased and healthy plants. I wish to record my thanks to Prof. W. Stiles for the necessary facilities to carry out the investigation in the Botanical Department of Birmingham University during the tenure of Research Scholarships awarded by the University. The work was carried out under the direction of Prof. C. G. C. Chesters, to whom I wish to express my sincere thanks for his aid and encouragement in the pursuance of research.

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A

Fig. 1.

B



Fig. 2.

EXPLANATION OF PLATE 14

Fig. 1. Healthy and diseased plants of the *Campanula* variety. A, healthy plant with extensive root system; B, plant at an early stage of the crown rot showing the discoloration of the crown region and its advance into the leaves and roots (see arrows). The root system appears weaker than that of the healthy plant because some of the roots have already been severed by the decay of the crown region.

Fig. 2. *Campanula persicifolia* plants from a pathogenicity experiment. The three plants in the front row have become diseased after soil inoculation while the plants behind them are healthy controls.

(Accepted for publication 6 November 1950)

GRAMINICOLOUS PYRENOMYCETES

I. THE CONIDIAL STAGE OF *TUBEUFIA HELICOMYCES*

BY JOHN WEBSTER

Department of Botany, University of Sheffield

(With 2 Text-figures)

Perithecia of *Tubeufia helicomyces* von Höhnelt are found on decaying grass stems growing in damp situations, frequently in association with a Hyphomycete, *Helicosporium phragmitis*. It has been shown that conidia referable to the latter fungus are formed in cultures prepared from ascospores of the former.

Perithecia of *Tubeufia helicomyces* von Höhnelt were collected on old decaying stems of *Arrhenatherum elatius* near the banks of the River Derwent above Stamford Bridge, York, on 27 August 1949, and again in the same locality on 10 November 1949. Specimens are preserved in the University of Sheffield Mycological Herbarium.

The perithecia are yellow in colour, turning brown with age, standing singly or in small groups on a brown subiculum. They are barrel-shaped with a distinct crown of stiff blunt bristles near the ostiole (Fig. 1A), and are 240–340 μ high by 180–230 μ in diameter. When dry the perithecia may collapse laterally, becoming somewhat flattened in a plane parallel to the long axis, or showing small longitudinal furrows in the perithecial wall.

The base of the perithecium bears numerous brown, septate, branched hyphae about 4 μ wide, attaching the perithecium to the subiculum. The wall of the body of the perithecium is made up of polygonal thin-walled cells about 15 μ wide. Above the crown of bristles the upper portion of the perithecium bears numerous rounded papillate cells projecting to form a dome above the bristles. The bristles themselves are stiff and septate 40–120 μ long, and taper from a somewhat bulbous base about 7–10 μ wide to a narrower rounded apex 4–5 μ wide (Fig. 1B).

The asci are numerous and are borne in a tuft near the base of the perithecium; they are club-shaped, tapering to a stalk at the base and possessing a rounded or bulbous apex, and measure 130–160 $\times$ 11–14 μ (ten measured in lactic acid). The wall of the ascus is distinctly double and contains 5–8 ascospores (Fig. 1C). The paraphyses are numerous, branched and filiform, somewhat longer than the asci, about 1 μ wide and swollen at the tip. The ascospores are needle-shaped, hyaline, septate with many large guttules and measure 66–150 $\times$ 2–3 μ —usually 90–110 $\times$ 2.5 μ (twenty measured in lactic acid) (Fig. 1D).

The perithecia of the fungus were closely associated with *Helicosporium phragmitis* von Höhnelt.

Cultures were prepared from the asci and ascospores of *Tubeufia* on 3 September 1949. Seven isolations were made, three from single ascospores

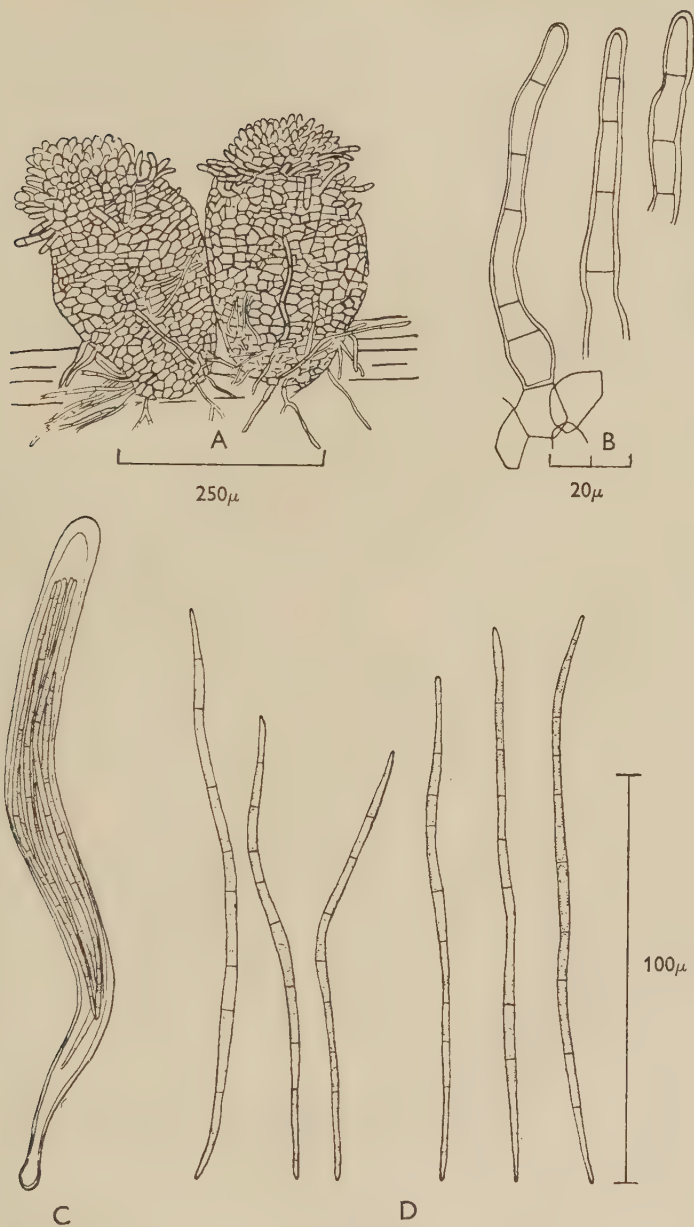


Fig. 1. A, perithecia of *Tubeufia helicomyces* (Herb. Sheffield no. 182). B, hairs from the apical crown of the perithecium. C, ascus. D, ascospores.

and four from single asci. The isolations were prepared by crushing a perithecium gently with a flamed needle in a drop of sterile water on a slide. The suspension of asci and ascospores so formed was drawn up into a fine sterile capillary pipette. The pipette was then touched lightly on to the surface of previously poured maize extract agar in a Petri dish, so that a number of drops of the suspension were deposited on the agar surface. The dish was then inverted, and each drop carefully examined under the low power of the microscope. The positions of drops containing single ascospores and single asci were marked with Indian ink. After two days the colonies resulting from the germination of single ascospores and from single asci were picked off. Transfers were made to tubes of oat agar and to stems of *Arrhenatherum* previously sterilized either by steaming or by exposure to propylene oxide vapour. The sterilized stems were placed with their lower ends buried in $1\frac{1}{2}$ in. of damp sand in boiling tubes. Conidia referable to *Helicosporium phragmitis* were produced within six weeks, in all cultures.

The conidial stage of this fungus growing with the perithecia on the collection from Stamford Bridge on *Arrhenatherum* occurs in small diffuse pearly grey patches which become faintly pink in colour as the spores are produced. The mycelium is branched and septate, and is made up of subhyaline hyphae $3-4\mu$ in width. The conidiophores are septate, branched or unbranched; they measure up to 200μ in length and are about 4μ wide at the base, tapering to 2μ wide at the apex. Conidia are formed on small, peg-like projections and are attached singly or in small groups to the conidiophore. From the projections the conidia develop as coiled branches (Fig. 2B, C). When fully mature the conidia are subhyaline and appear as flat spirals with four or five tight coils. The spores contain a number of guttules and 3-11 transverse septa, and measure $14-18\mu$ in diameter, the thickness of the coil being $1-1.5\mu$ (Fig. 2A).

In culture the fungus grows rather slowly. On oat-agar slopes at room temperature and in diffuse light, colonies about 2 cm. long are formed after six weeks. A closely growing mat of grey or buff-coloured mycelium is formed, which takes on a pink colour as conidia are produced. From the aerial mycelium numerous conidiophores are formed, which are often much longer than those found on grasses. Conidiophores as long as 520μ have been observed. Numerous conidia are produced. These are similar in every way to those collected with the perithecia, but are slightly smaller, measuring $12-16\mu$ in diameter.

Perithecia of *Tubeufia* have also been collected on other hosts, and specimens have been deposited in the University of Sheffield Mycological Herbarium as follows:

No.	Host	Locality	Date
812	<i>Glyceria</i>	Wheatfen Broad, Norfolk	15 June 1950
813	<i>Glyceria</i>	Wheatfen Broad, Norfolk (coll. E. A. Ellis)	16 June 1950
814	<i>Phragmites</i>	Wheatfen Broad, Norfolk	20 June 1950

The conidial stage of this fungus occurs commonly throughout the year on stems and leaf-sheaths of various grasses and cereals, especially when these occur in damp locations. Various specimens of this fungus on a number

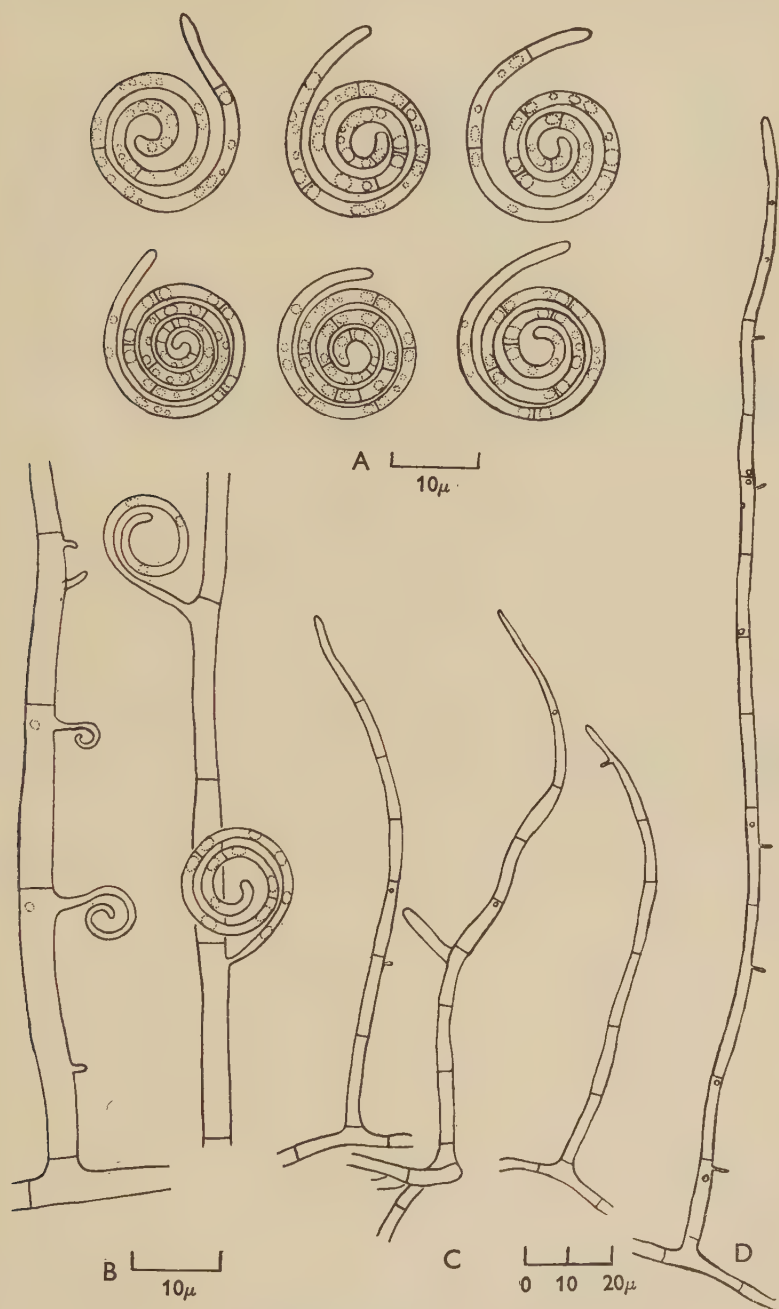


Fig. 2. A, conidia produced in a culture on oat agar derived from a single ascospore of *Tubeufia helicomyces*. B, formation of conidia. C, conidiophores from *Arrhenatherum* (Herb. Sheffield no. 182). D, conidiophore produced in cultures on oat agar.

of hosts have been collected and are in the University of Sheffield Mycological Herbarium:

No.	Host	Locality	Date
197	<i>Triticum</i>	Burton Constable, East Yorks	23 March 1949
198	<i>Dactylis</i>	Cottingham, East Yorks	20 October 1948
199	<i>Agropyron</i>	Grounds of University College, Hull	13 July 1949
247	<i>Avena</i>	Ringinglow, nr. Sheffield	22 March 1950
268	<i>Hordeum</i>	South Wootton, King's Lynn, Norfolk	1 April 1950
389	<i>Triticum</i>	Holmesfield, Derbyshire	24 May 1950
395 ^b	<i>Dactylis</i>	Annesley Park, Notts	29 May 1950
811	<i>Phalaris</i>	Wheatfen Broad, Norfolk	18 June 1950

Helicosporium phragmitis was described by von Höhnelt (1905) on rotting culms of *Phragmites communis* in association with *Acanthostigmella genuflexa*, and it was suggested that the two fungi might be connected. The cultural investigations described above demonstrate, however, that *Helicosporium phragmitis* is the imperfect stage of *Tubeufia helicomyces*.

REFERENCE

HÖHNEL, F. von (1905). Mycologisches Fragmente. *Ann. mycol., Berl.*, **3**, 338.

(Accepted for publication 24 October 1950)

NOTE ADDED JULY 1951

Since this paper went to press Ellis *et al.* (1951) have described the occurrence of *H. phragmitis* on various hosts, frequently in association with *Tubeufia helicomyces*.

REFERENCE

ELLIS, M. B., E. A. & J. P. (1951). British marsh and fen fungi. *Trans. Brit. mycol. Soc.* **34**, 147-69.

GRAMINICOLOUS PYRENOAMYCETES

II. THE OCCURRENCE OF THE PERFECT STAGE OF
HELMINTHOSPORIUM TERES IN BRITAIN

By JOHN WEBSTER

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(With Plates 15 and 16, and 2 Text-figures)

A form of *Pleospora trichostoma* occurs widely on barley stubble in Britain. Conidia referable to *Helminthosporium teres* Sacc., produced together with the perithecia and in culture, are genetically connected with it. In infection experiments the fungus produced 'netblotch' symptoms on the leaves of barley seedlings. Previous records of the fungus on barley are given.

The perfect stage of *Helminthosporium teres* Sacc., the causal organism of 'netblotch' of barley, is believed to be of rare occurrence in Britain (*Common Plant Diseases*, 1944; Butler & Jones, 1949). Records in other countries are given below.

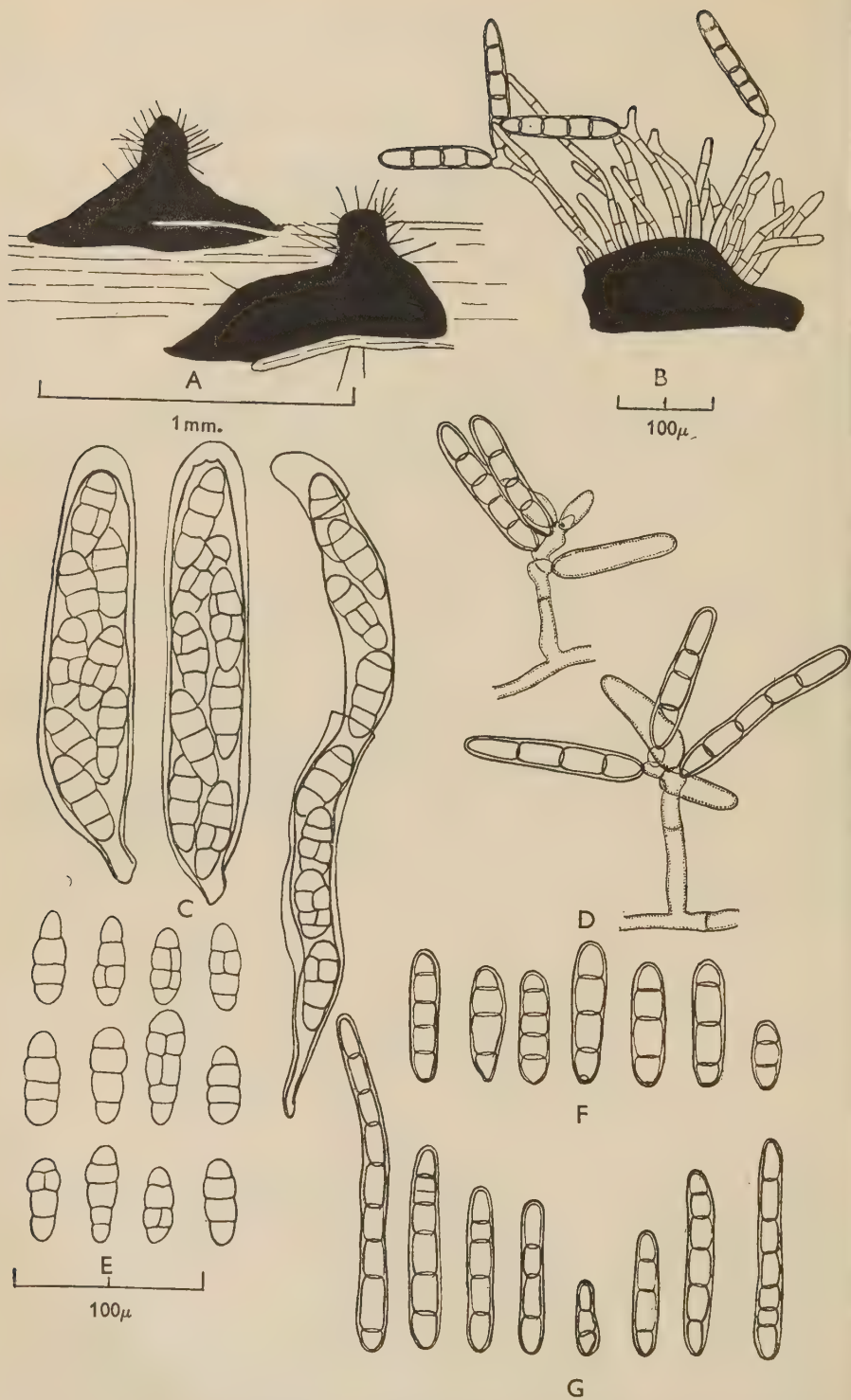
During 1949 and 1950, several collections were made of a Pyrenomycete on barley stubble. Cultures prepared from the ascospores yielded conidia of the *Helminthosporium* type. When these conidia were inoculated on to leaves of barley seedlings, symptoms of 'netblotch' appeared.

Specimens studied. The following specimens have been collected and are deposited in the Mycological Herbarium of the University of Sheffield. Duplicates of some of these specimens have been sent to the Herbarium of the Plant Pathology Laboratory, Harpenden, Herts, and the Royal Botanic Gardens, Kew.

No.	Locality	Date
180	South Cave, Yorks	27 March 1949
233	South Cave, Yorks	19 March 1950
170	Skidby, nr. Beverley, Yorks	26 Feb. 1950
228	Skidby, nr. Beverley, Yorks	18 March 1950
248	Skidby, nr. Beverley, Yorks	25 March 1950
230	Little Weighton, nr. Beverley, Yorks	19 March 1950
249	King's Lynn, Norfolk	1 April 1950
250	East Kirkby, Notts	9 April 1950
251	East Kirkby, Notts	9 April 1950

The collection made at South Cave consisted of black sclerotium-like bodies growing on barley stubble of the previous season among clover. When portions of the stubble were placed in a Petri dish on damp sand these bodies gave rise to numerous conidiophores, bearing conidia of the *Helminthosporium* type (Text-fig. 1 B, F). Conidiophores were also observed to arise directly from the surface of the stubble.

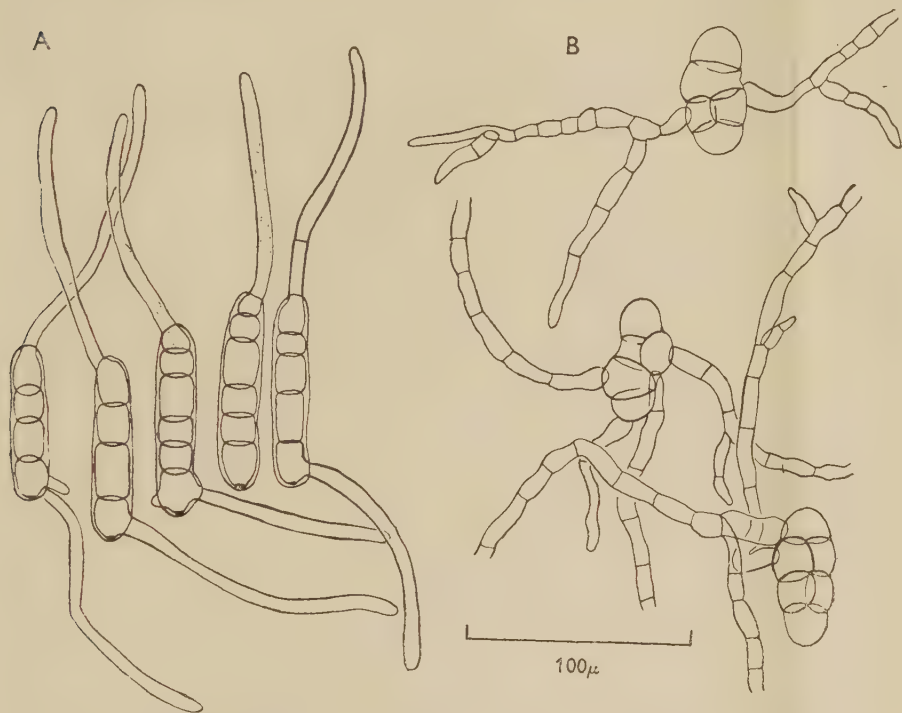
After a few days, beak-like processes, surrounded by a distinct fringe of stiff black hairs, developed on certain of the sclerotia (Text-fig. 1 A); sometimes two such beaks were observed on a single sclerotium (Pl. 16, fig. 2).



Text-fig. 1. *Pleospora trichostoma*, Herb. Sheffield no. 180. A, perithecia on barley stubble; B, sclerotium on barley stubble, bearing conidiophores; C, asci; D, conidiophores from culture on tap-water agar; E, ascospores; F, conidia from barley stubble; G, conidia from culture.

Examination of these 'beaked sclerotia' showed that they contained asci with three to eight ascospores (Text-fig. 1 C; Pl. 15, fig. 1).

The young sclerotia develop beneath the epidermis, bursting through as elongate black bodies. At maturity these bodies develop a conical or cylindrical beak and measure $430\text{--}770\ \mu$ long, $200\text{--}300\ \mu$ wide and $380\text{--}430\ \mu$ high (eight measured). The beak and other portions of the sclerotium wall bear stiff, black, thick-walled, septate, tapering hairs, $140\text{--}225\ \mu$ long, $8\text{--}10\ \mu$ wide at the base and tapering to $4\text{--}5\ \mu$ at the rounded tip (ten measured) (Text-fig. 1 A).



Text-fig. 2. *Pleospora trichostoma*, Herb. Sheffield no. 180. A, conidia germinating after 2 hr. on maize extract agar; B, ascospores germinating after 24 hr. in tap water.

The asci are broadly club-shaped or cylindrical, with short stalks and rounded apices. The wall is distinctly double; rupture of the outer wall and extension of the thin inner wall may be readily observed (Pl. 15, fig. 1; Text-fig. 1 C). The asci measure $175\text{--}235 \times 38\text{--}40\ \mu$ (ten unbroken asci measured in lactic acid). The number of ascospores varies from three to eight per ascus. In asci with five to eight spores the spores are usually biseriate; where fewer spores are present they are usually uniseriate.

The ascospores have three to four transverse septa (usually three) and up to two longitudinal septa (Text-fig. 1 C), and are pale yellow in colour. The two central cells are frequently somewhat larger than the rounded end-cells, and there is a slight constriction at the septa. The ascospores measure $36\text{--}51 \times 14\text{--}20\ \mu$ (twenty measured in lactic acid).

Cultures were prepared from single ascospores, single asci and single conidia. Both types of spore germinated readily, germ tubes being produced within a few hours. The ascospores produced germ tubes usually from the central cells, but occasionally from the end-cells. The germ tubes are 8–10 μ wide, septate and branched. The conidia extruded one or two germ tubes from the end-cells of the conidium. The germ tubes were about 6 μ wide, vacuolate, becoming septate and branching (Text-fig. 2 A, B). The cultures, from both ascospores and conidia, were similar in every way. When inoculated to tap-water agar as recommended by Drechsler (1923), conidia of the *Helminthosporium* type were produced within ten days (Pl. 15, fig. 2; Text-fig. 1 D, G). When inoculated to richer media (e.g. oat agar) conspicuous grey tufts of hyphae were formed, reaching the top of the Petri dish (Pl. 16, fig. 1). In old cultures sclerotia were formed, bearing stiff, black hairs. Asci were not found within these bodies. Pycnidia were not observed.

A comparison of the conidia produced in cultures derived from ascospores with the conidia formed on the stubble showed that they were essentially similar in shape and colour, but differed somewhat in range of size and number of septa. The conidia produced on the sclerotia on the stubble were borne on conidiophores 60–230 μ long. These conidia were cylindrical, with rounded ends, or tapering slightly from the base, with two to five transverse septa, not constricted at the septa, pale yellow to olive green in colour, and measured 43–80 $\times$ 11–18 μ (twenty measured in lactic acid).

Conidia produced in cultures on tap-water agar were usually borne on shorter conidiophores (up to 120 μ long). They contained none to eight transverse septa, and measured 54–180 $\times$ 14–19 μ . In culture, proliferation of the conidia at the apex was occasionally observed (Pl. 15, fig. 2). Conidia from material and from cultures are compared in Text-fig. 1 G, and measurements of conidia on stubble and in culture are given in Table 1 for this and other collections of the same fungus. These other collections agree in all essential details with the original South Cave collection. Examination of the further range of material, however, showed that stiff hairs are not invariably present on the sclerotia. Occasionally completely

Table 1

Herb. effield cimen no.	Size of perithecia (μ)			Size of asci (μ)	Ascospores		Conidia	
	Length	Breadth	Height		Size (μ)	No. of septa	Size (μ)	No. of septa
180	430–770	200–240	380–430	174–235 $\times$ 38–40	36–51 $\times$ 14–20	3–4 $\times$ 0–2	(a) 43–80 $\times$ 11–18 (b) 54–180 $\times$ 14–19	2–5 0–8
170	420–900	360–480	240–400	185–254 $\times$ 30–38	40–52 $\times$ 16–22	2–4 $\times$ 0–2	(a) 45–90 $\times$ 15–17 (b) 32–74 $\times$ 14–22	3–5 0–4
230	600–780	180–350	180–350	206–270 $\times$ 30–40	42–54 $\times$ 16–22	3 $\times$ 1–3	(a) 35–90 $\times$ 15–18 (b) 36–90 $\times$ 12–18	2–5 0–6
233	540–720	240–300	230–360	195–245 $\times$ 35–42	38–49 $\times$ 16–20	3 $\times$ 0–2	(a) 64–96 $\times$ 14–18*	3–7
251	600–900	180–420	180–230	140–220 $\times$ 35–38	38–55 $\times$ 16–22	3 $\times$ 0–2	—	—
249	600–840	250–300	180–300	140–210 $\times$ 38–50	34–50 $\times$ 17–20	3 $\times$ 0–2	—	—
228	420–840	240–420	240–480	170–250 $\times$ 30–46	46–52 $\times$ 15–19	3 $\times$ 0–3	—	—

Failed to form conidia in culture.

(a) Conidia from stubble.

(b) Conidia formed in culture.

Measurements made from ten asci, twenty ascospores, and twenty conidia in lactic acid.

naked sclerotia are found, containing asci. Further, the conspicuous conical or cylindrical papilla present in the original South Cave collection was not always found in the other collections. Occasionally the end-cell of certain of the ascospores contained a longitudinal septum.

Cultures were also prepared from certain of these collections (170, 230, 233). In all except no. 233 conidia were produced. Various attempts to produce conidia from the isolations of collection no. 233 failed.

INFECTION EXPERIMENTS

Attempts were made to inoculate barley seedlings with conidia produced in culture. Three varieties were used: Hunter's Plumage Archer, Hunter's Spratt Archer and seed of an unknown variety. The seeds were first rinsed in alcohol, immersed for 1 min. in 0.01 % solution of mercuric chloride, and then thoroughly rinsed in sterile water. Treated seeds were placed on damp filter-paper in sterile Petri dishes to germinate. After germination the seedlings were potted in pairs in small pots. When the seedlings had reached the first leaf stage they were inoculated by brushing conidia over the surface of the leaf and coleoptile. The conidia were picked up from the culture on the damp tip of a camel-hair brush under a binocular dissecting microscope. Care was taken to ensure that conidia had actually been transferred. Following inoculation the seedlings were moved to a cool greenhouse, and kept under humid conditions for 2 days by placing the pots under a large glass case. Controls were prepared in a similar manner, but the leaf and coleoptile were brushed with sterile water instead of conidia. After 2 days the glass case was removed.

Within 7 days lesions were observed on certain of the inoculated seedlings. The lesions were yellow to brown in colour, 12–20 mm. long, occasionally extending across the whole of the leaf lamina and showing more or less conspicuous darker brown reticulate markings (Pl. 15, fig. 3). All the controls remained healthy. Isolations were made from lesions on inoculated plants by cutting infected leaves into small pieces with flamed scissors, rinsing in sterile water, and then plating the leaf fragments on to tap-water agar. Within 7 days *Helminthosporium* conidia were produced in these cultures. Table 2 gives the results of inoculation experiments with three isolations from ascospores and three varieties of barley.

Table 2

Collection no.	No. of seedlings inoculated	No. of seedlings infected	Controls, no. of seedlings	No. of seedlings infected
180	12 U	9	10 U	0
170	14 P	1	12 P	0
	12 S	6	3 S	0
230	20 P	9	10 P	0

U = variety of barley unknown.

P = Hunter's Plumage Archer.

S = Hunter's Spratt Archer.

As conidia were not produced in isolations from collection 233, attempts were made to inoculate barley seedlings with agar disk inocula of these

isolates, but without success. Attempts to infect seed from conidia of the other isolates were also unsuccessful.

From the type of lesion produced it is concluded that we are here dealing with the 'netblotch' organism, *H. teres* Sacc. This conclusion is supported by cultural evidence. Various writers (Drechsler, 1923; Ito, 1930; Ito & Kuribayashi, 1931) have commented on the characteristic tufted growth of this fungus in culture. Moreover, whereas *H. teres* will readily produce conidia in culture, *H. gramineum* Rabenh. will not (Johnson, 1925; Ito & Kuribayashi, 1931). Houston & Oswald (1946), however, have shown that *H. gramineum* will produce conidia in culture under suitable conditions.

The perithecial stage of the fungus is clearly a form of *Pleospora trichostoma* (Fr.) Ces. & de Not., which is regarded by Wehmeyer (1949) as a 'species complex', in which is included various groupings previously accorded specific rank (e.g. *Pyrenophora teres* Drechsler).

EARLIER RECORDS

Before considering *Pyrenophora teres* in detail, it is relevant at this stage to review previous records of perithecia of the *Pleospora trichostoma* group on barley.

Ravn (1900) seems to have been the first to suggest the association of *Helminthosporium teres* with a member of this group. He succeeded in producing sclerotia on sterilized barley straw, and although he failed to observe asci in these bodies, he suggested that they might be immature perithecia of a Pyrenomycete, possibly *Pleospora polytricha* (Wall.) Tul., which is included in the *P. trichostoma* group by Wehmeyer (1949).

Diedicke (1903) collected a *Pleospora* (similar to the forms of *P. trichostoma* which he had previously described in 1902) on stubble of barley which in the previous summer had been severely infected with *Helminthosporium gramineum*. He described experiments in which barley seedlings were inoculated with crushed perithecia. Of sixteen plants treated in this manner six became infected and showed symptoms of 'leaf stripe' (Streifenkrankheit). To this fungus he gave the name *Pleospora graminea*. He further suggested that *Helminthosporium teres* would be found to have a *Pleospora* as its ascigerous stage, and he proposed that this, as yet undiscovered, fungus should be named *P. teres*. Noack (1905) stated that the perfect stage of *Helminthosporium gramineum* was *Pleospora trichostoma*, of which he gave a detailed description. The perithecia were usually 780–800 μ long, bearing stiff black bristles. Measurements of asci are not given, but from a drawing and from the stated magnification, a single ascus was calculated to measure $201 \times 39 \mu$. Two forms of ascospores were distinguished; a broad form up to 44 μ long and 20 μ wide, and a narrow form 49–54.5 μ long $\times$ 17–20 μ wide. The production of conidia in a culture derived from an ascospore is described.

A. G. Johnson (1914) recorded the discovery at Madison, U.S.A., of a *Pleospora* on old barley straw, but did not describe the collection in detail. A culture was prepared from a single ascus: 'A pure colony resulted which showed the characteristic mycelial growth of *Helminthosporium teres*. From this colony and from transfers from it typical *H. teres* conidia were obtained.

They were used to inoculate twelve healthy barley plants under glass. Typical *H. teres* conidia were obtained. They were used to inoculate twelve healthy barley plants under glass. Typical *H. teres* lesions resulted on all.'

Paxton (1922) claimed to have collected the mature perithecial stage of *H. gramineum* on 2-year-old barley straw in California. Cultures were prepared and 'typical *H. gramineum* conidia were produced. *Hordeum sativum* inoculated with the same gave typical lesions of this species.'

Poeteren (1922) found 'perithecia of *Pleospora trichostoma* on the glumes of germinated grain of barley the seedlings of which were affected by stripe disease (*Helminthosporium gramineum*)'.

Vogt (1923) described perithecia of *Pleospora trichostoma* on diseased barley stems, and concluded that he had the perfect stage of *Helminthosporium gramineum*.

Drechsler (1923) described under the name *Pyrenophora teres* (Diedicke) Drechsler* the ascigerous stage of *Helminthosporium teres*, collected at Madison, U.S.A., in 1919 (that is, in the same locality as the earlier collection by Johnson in 1914). The measurements given for the asci are $230-250 \times 30-36 \mu$, and for the ascospores $50-60 \times 18-22 \mu$. He pointed out that the fungus was very similar to the one described by Diedicke (1903) and Noack (1905). He also commented that 'the work of both Diedicke and Noack is open to the criticism that it was not done in connection with parallel infection experiments carried out with *Helminthosporium teres*, and that consequently these authors were perhaps not so capable of distinguishing between the pathological effects of the two related fungi as might have been desired'.

Ito (1930) described *Pyrenophora graminea* Ito & Kuribayashi as the ascigerous stage of *Helminthosporium gramineum* on *Hordeum sativum* in Japan (the combination *Pyrenophora graminea* (Died.) Drechsler, cited by Brooks (1928), was not made by Drechsler, and is therefore invalid). The Japanese fungus is, however, quite distinct from the fungus with which Diedicke and Noack worked, and which is presumably identical with the fungus recorded by Paxton (1922), Poeteren (1922), and Vogt (1923) as the ascigerous stage of *Helminthosporium gramineum*. The description of the Japanese specimen shows that the asci measure $225-425 \times 32-50 \mu$, while the ascospores measure $45-75 \times 20-32.5 \mu$, which is appreciably larger than the size usually recorded for the *Pleospora trichostoma* group. In a later paper Ito and Kuribayashi (1931) comment on this discrepancy, and express doubts on the results of Diedicke, Noack and Paxton. 'The measurements of ascospores and the production of the conidia on cultural media clearly show that Noack's fungus is *Pyrenophora teres*. In 1922, Paxton also reported the formation of conidia, when the ascospores were transferred on corn meal agar. Hara (1925) collected a species of *Pyrenophora* on barley-straw and identified it as *Pyrenophora trichostoma* (Fr.) Sacc., taking it as the ascigerous stage of *H. gramineum*. He gave the measurements of the ascospores as $44-55 \times 18-21 \mu$, which rather correspond to those of *P. teres*. *Pyrenophora trichostoma* is often considered to be the perithecial stage of *H. gramineum*, but it differs from our fungus by the smaller size of the ascospores ($44-50 \times 17-20 \mu$ by Winter, while $45-75 \times 20-32.5 \mu$ in ours). After comparison

* The correct citation is *Pyrenophora teres* Drechsler (Wakefield & Moore, 1936).

with the related species, we have decided finally that our fungus is the real ascigerous stage of *H. gramineum*.'

In Table 3 the measurements given by Drechsler for *Pyrenophora teres* are compared with the total range of measurements found in the present collections, and with the measurements given by Wehmeyer for his *Pleospora trichostoma* group. It will be seen that the size of the ascospores of the present collections is somewhat smaller than those found by Drechsler in his material. In spite of this, taking into consideration the cultural evidence and the results of infection experiments, I conclude that we are dealing with the same fungus, but prefer to adopt the course chosen by Wehmeyer and to use the name *P. trichostoma* for this fungus. *P. trichostoma* has been recorded in Britain on several occasions (Bisby & Mason, 1940), but apparently not on barley.

Table 3

Name applied to fungus	Authority	Size of asci	Size of ascospores (μ)	Size of conidia (μ)
<i>Pyrenophora teres</i>	Drechsler	220-250 $\times$ 30-36	50-60 $\times$ 18-22	30-175 $\times$ 15-22
<i>Pleospora trichostoma</i> (collections entered in Table 1)	—	140-270 $\times$ 30-50	34-55 $\times$ 14-22	35-96 $\times$ 11-18
<i>Pleospora trichostoma</i>	Wehmeyer	160-210 $\times$ 35-50	44-60 $\times$ 18-25	—

The widespread occurrence of perithecia of this fungus on barley stubble in the spring raises the problem of whether such stubble is a serious source of infection of seedlings. Without further experimental work it is not possible to answer the question, but infected grain is probably the most frequent source of infection.

I wish to thank the Director of the Royal Botanic Garden, Kew, for permission to study literature and specimens, and to Miss Wakefield and Dr Dennis of the same establishment for helpful discussion. My thanks are also due to Mr E. Barron for his assistance with the photography, and to Mr N. L. Johnson for help with some of the inoculations.

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Fig. 1.



Fig. 2.



Fig. 3.

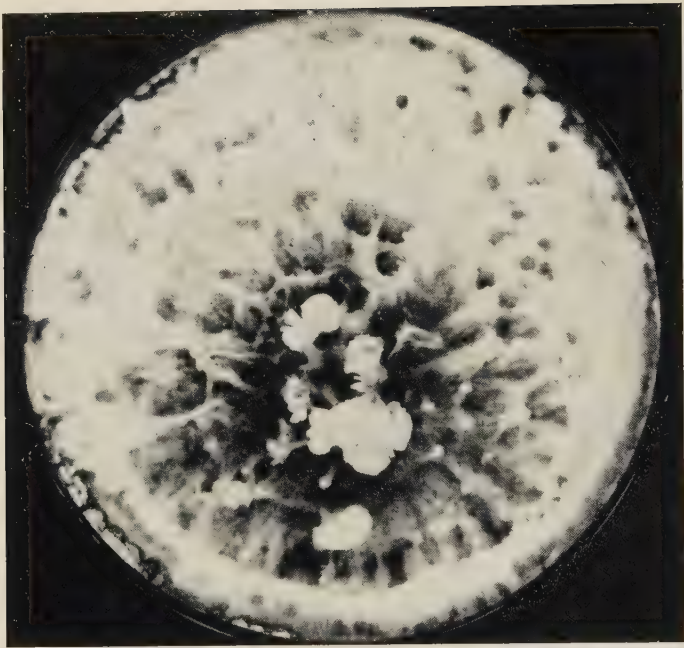


Fig. 1.

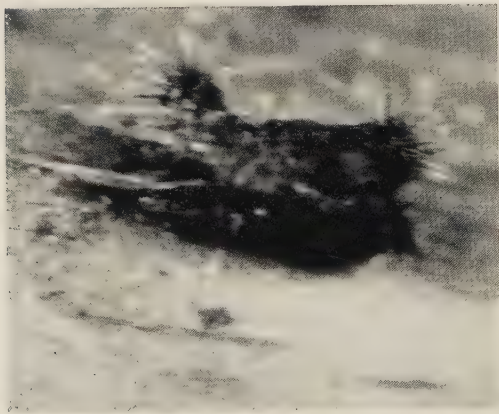


Fig. 2.

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EXPLANATION OF PLATES 15 AND 16

PLATE 15

- Fig. 1. Asci of *Pleospora trichostoma*, $\times 370$. The double ascus wall can clearly be seen in the ascus on the right. The outer wall has ruptured.
- Fig. 2. Conidia formed in culture derived from a single ascospore, after 10 days on tap-water agar, $\times 370$. The formation of secondary conidia is shown.
- Fig. 3. Barley seedlings infected 10 days previously with conidia of *Pleospora trichostoma*, showing the lesions formed on the leaves. Natural size.

PLATE 16

- Fig. 1. Culture of *Pleospora trichostoma* on oat agar, showing the characteristic tufted growth.
- Fig. 2. 'Sclerotium' of *Pleospora trichostoma* on barley stubble, $\times 50$, showing the formation of two papillae, fringed with black hairs.

(Accepted for publication 10 November 1950)

GRAMINICOLOUS PYRENOMYCETES

III. THE OCCURRENCE OF *MICROTHYRIUM CULMIGENUM*
ON GRASSES IN BRITAIN

By JOHN WEBSTER

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(With Plate 17 and 1 Text-figure)

Microthyrium culmigenum Sydow is recorded for the first time in Britain, where it is apparently common on stems and leaf sheaths of grasses. Stages in development of the thyriothecia are briefly described and illustrated.

When making collections of Pyrenomycetes occurring on grasses and cereal stubble a *Microthyrium* was commonly found which matched very closely the description and type specimen of *M. culmigenum* Sydow. Although common, this fungus has not, apparently, been previously recorded in this country, and the purpose of this paper is to describe it.

Specimens studied. The following specimens have been collected and are deposited in the Mycological Herbarium of the University of Sheffield:

No.	Host	Locality	Date	Collector
800	<i>Arrhenatherum</i>	Stamford Bridge, York	10 Sept. 49	N. H. Brittan
145	<i>Arrhenatherum</i>	Londesborough, East Yorks	25 June 49	J. Webster
802	Oat stubble	East Kirkby, Notts	26 Aug. 49	J. Webster
801	<i>Dactylis</i>	Stamford Bridge, York	10 Sept. 49	N. H. Brittan
881	<i>Dactylis</i>	Stamford Bridge, York	27 Aug. 49	J. Webster
818	<i>Dactylis</i>	Fulwood, Sheffield	11 July 50	J. Webster
883	<i>Dactylis</i>	Castle Howard, Malton, Yorks	23 Sept. 50	J. Webster
884	<i>Dactylis</i>	Annesley Park, Notts	21 Sept. 50	J. Webster
979	<i>Dactylis</i>	Fulwood, Sheffield	29 Oct. 50	J. Webster
980	<i>Holcus mollis</i>	Fulwood, Sheffield	29 Oct. 50	J. Webster

In addition to the specimens cited above part of the type collection has been studied. This collection is in Herb. I.M.I. (33955), and is part of an exsiccatum issued by Petrak as: 'F. Petrak, Mycotheca generalis, 1325, on *Calamagrostis lanceolata* Brandenburg: Rangsdorf bei Zossen, leg H. Sydow. VIII, 1918.'

It is not to be concluded that the fungus occurs more frequently on *Dactylis* than on other hosts. The more numerous collections on *Dactylis* are partly the result of a particular study at present being made of the micro-fungi on that host. Parts of some of these collections have been sent to the Herbarium of the Commonwealth Mycological Institute, Kew, and the Herbarium of the Royal Botanic Gardens, Kew.

The British collections consist of stems and leaf-sheaths bearing small black thyriothecia. Preparations made from young material on *Dactylis* stems by the modified 'Necol' technique described by Ellis (1950) show that there is, at first, an abundant superficial mycelium, made up of

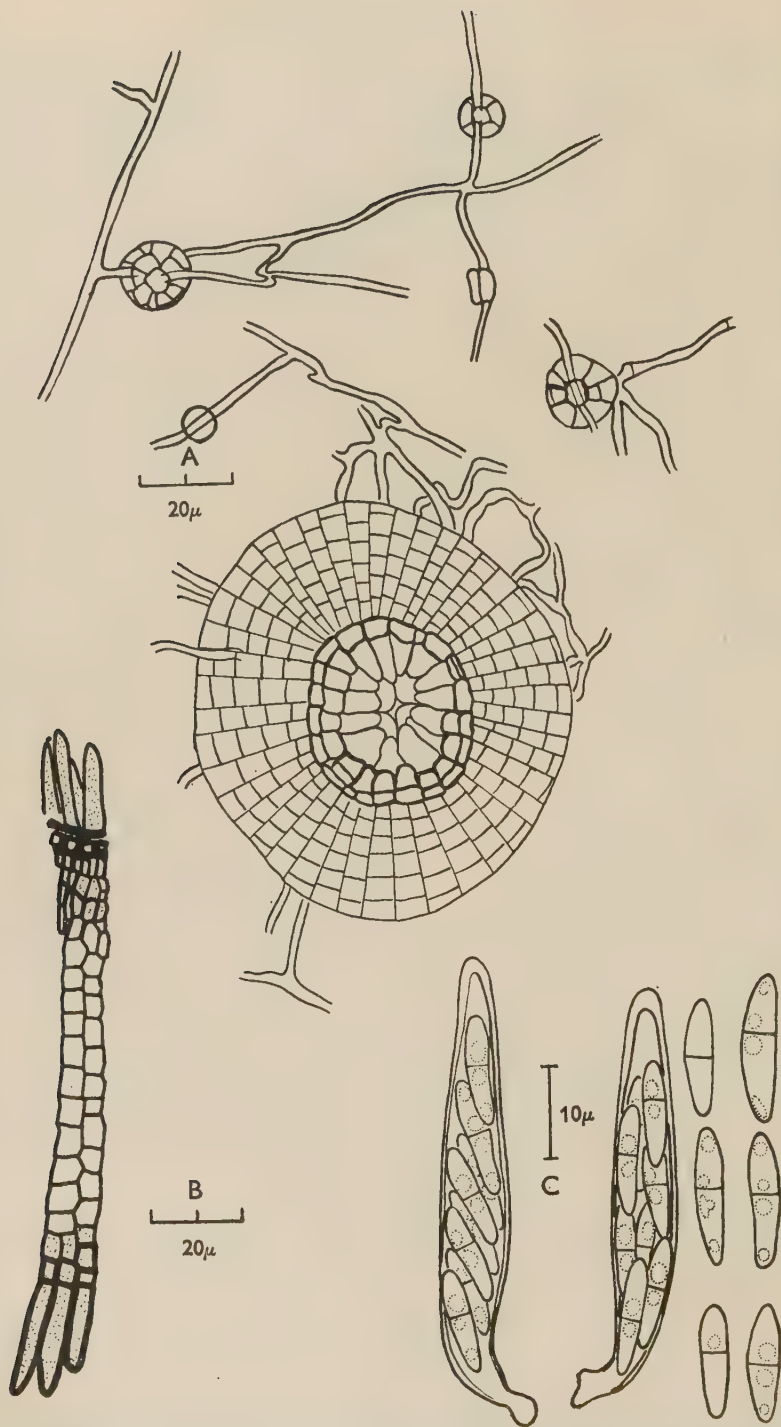
branched, anastomosing, hyaline hyphae $2-3\mu$ wide. The thyriothecia develop from intercalary cells of the mycelium. The details of development have not been followed, but a number of stages are illustrated in Text-fig. 1. The intercalary cell enlarges considerably and probably divides by a septum in a plane parallel to the substratum. The upper cell so formed gives rise to the upper wall (the covering membrane) of the thyriothecium, the lower cell to the asci, the basal membrane and to certain sterile hyphae which emerge from beneath the covering membrane (Text-fig. 1A). By the addition of further septa in the upper cell a radial symmetry is soon attained, and development proceeds centrifugally. Mature thyriothecia are crowded together and sometimes confluent or occasionally scattered, black, circular in outline, distinctly conical with a conspicuous thickened ring surrounding the ostiole, and are somewhat variable in size, measuring from 120 to 200μ in diameter (Pl. 17, fig. 2). The covering membrane is made up of radiating files of approximately square cells about $6-8\mu$ wide, with thin dark walls, the files running out as the margin into bluntly pointed processes (Text-fig. 1B). Near the centre the cells are smaller and thick-walled, and around the ostiole is a collar of very thick-walled cells. The collar is perforated at intervals by a series of holes which penetrate the wall of the thyriothecium. Above the perforations in the collar is a rim, crowned by a number of stiff, thick-walled, tapering 'bristles'—about 15μ long and 4μ wide at the base (Text-fig. 1B). The differentiation of the cells surrounding the ostiole occurs quite early in the development (Text-fig. 1A). The 'bristles' are not invariably present. They are regarded as the innermost cells of the radial files, which have separated from each other. The thyriothecia contain numerous asci which are obclavate and sessile or occasionally very shortly stalked. The asci are wider near the base, and taper to the rounded apex, the wall is distinctly double. The asci measure $35-50\mu$ long and $7-8\mu$ wide (ten measured in lactic acid) and contain eight spores. Paraphyses were not seen. The ascospores are irregularly arranged within the ascus, and may be quadriseriate; they are cylindrical to club-shaped, with rounded or somewhat pointed ends, hyaline, guttulate, with a single septum dividing the spore into two usually unequal portions, and measure $10-13 \times 2-3\mu$ (twenty measured in lactic acid).

Preparations were made from the type collection by the modified 'Necol' technique. Little superficial mycelium was observed. The thyriothecia agreed with the description given above, and, in particular, the characteristic collar with perforations and 'bristles' could be clearly distinguished. Although no asci or ascospores were seen I have no doubt that all the collections cited above belong to this species.

The measurements given by Sydow (1921, p. 140) for this species, and the measurements of the British collections are very similar:

Specimen	Thyriothecia (μ)	Asci (μ)	Ascospores (μ)
Type	150-180	$35-48 \times 8-10$	$10-15 \times 3-3.5$
Sheffield no. 800	120-200	$36-50 \times 7-8$	$10-13 \times 2-3$

There are few other records of members of the Microthyriaceae occurring on grasses in Britain. Grove (1933) described *Microthyrium gramineum* var.



Text-fig. 1. *Microthyrium culmigenum*. A, drawings made from 'Necol' preparations, showing the superficial mycelium and developing thyriothechia. Differentiation of the 'collar' cells has begun in the largest developing thyriothecium. B, a sector of the covering membrane of a mature thyriothecium, showing at the centre the structure of the 'collar' and the 'bristles' surrounding the ostiole. C, asci and ascospores.



Fig. 1.

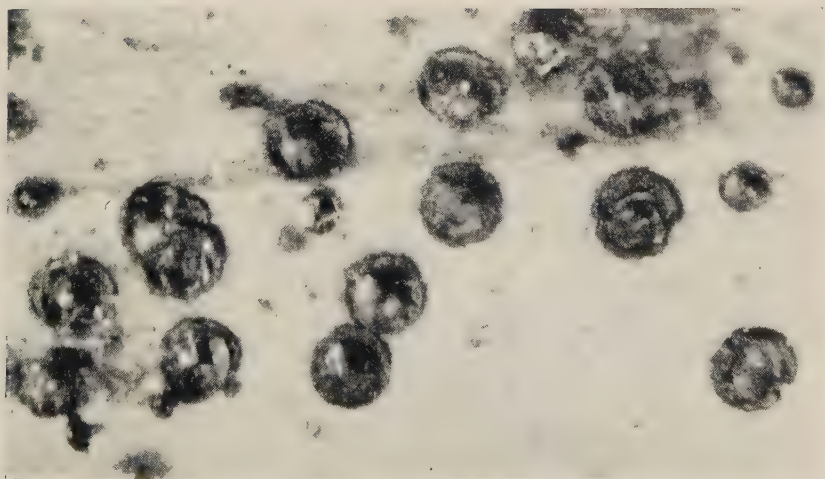


Fig. 2.

major on *Ammophila*, from Kent. From the description this fungus would seem to be rather larger than *Microthyrium culmigenum*, with thyriothechia 300–400 μ in diameter, asci 70–80 μ long $\times$ 7–8 μ wide, and ascospores 14–15 μ long and 4–5 μ wide.

I acknowledge with grateful thanks the helpful criticism and advice received from Dr M. B. Ellis of the Commonwealth Mycological Institute. My thanks are also due to Mr E. Barron for his assistance with the photography.

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EXPLANATION OF PLATE 17

Fig. 1. *Microthyrium culmigenum*. Covering membrane. $\times 390$.

Fig. 2. Thyriothechia on surface of *Dactylis* stem. $\times 60$. The 'collar' surrounding the ostiole can clearly be seen in the lower left corner of the photograph.

THE ECOLOGY OF PREDACIOUS FUNGI

I. PRELIMINARY SURVEY

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An account is given of the occurrence of predacious fungi on various kinds of substrata from different parts of Britain, and the results of a number of collections are tabulated, to indicate what species have been found in the different types of material investigated. An analysis of 135 collections shows that they are particularly abundant among bryophytes. The distribution of thirty-four species of predacious fungi on different substrata is tabulated, and the use of quadrats in studying their distribution is described.

INTRODUCTION

More than a hundred species of predacious fungi are known, mainly through the work of Drechsler in America, and the British flora includes nearly fifty species, with unquestionably many more yet to be recorded. Up to the present time most of the studies on these fungi have been morphological, and no serious attempt has been made, either here or abroad, to investigate the ecology of this interesting group.

The purpose of this paper is to give a preliminary survey of distribution of predacious fungi in different kinds of material from various parts of Britain.

THE HABITAT OF PREDACIOUS FUNGI

Amoebae and nematode worms are the principal hosts of the predacious fungi, and these fungi are normally to be found wherever these animals occur. Certain habitats, however, seem to be better suited to these fungi than others, irrespective of the numbers of host animals present; conversely, the presence of large numbers of nematodes or of amoebae in a given collection is by no means a guarantee that predators will be found. It seems likely, therefore, that the habitat has a direct effect on the fungi themselves apart from its influence on the animals serving as prey. Precisely how the habitat exerts this influence is as yet far from clear.

The principal terrestrial habitats that have been investigated in the course of the present work are: leaf mould, decaying wood, partly decayed plant remains, dung, and living bryophytes. The soil is not included in this list, but it must be remembered that most of the materials examined have been collected from on or near the soil, and it is possible that the soil itself may be the ultimate reservoir of many of the predacious fungi. Aquatic forms are also excluded from the list, although, apart from such true aquatic types as *Zoophagus insidians* Sommerstorff, first recorded from Britain by Barnes and Melville (1932), it has been shown by Peach (1948, 1950) that a number of species normally regarded as terrestrial may be obtained from aquatic habitats.

Leaf mould. Forest leaf mould was used extensively as a source of material during the earlier part of the work, but results were, on the whole, disappointing. Various species were recorded, but many cultures showed no sign of predators, although nematodes and amoebae were present in abundance. This may have been because crumbly, well-rotted leaf mould was most often used, whereas later experience has shown that portions of decaying leaf and twig buried in leaf mould are more fruitful.

Decaying wood. This has proved to be a most fruitful source of predacious fungi, especially of the hyphomycetous types. Wood in an advanced stage of decay, such as old tree-stumps and dead branches with bark peeling off, is often rich in nematodes, and also appears to be a substratum on which predacious fungi are able to grow, judging from the freedom with which they sporulate on small pieces of decomposing wood in laboratory cultures.

Partly decayed plant remains. Plant material of various kinds decaying on or near the soil is often rich in predacious fungi, especially leaves and herbaceous stems which are just entering the 'mushy' stage of decomposition. Portions of stem and leaf that have lain on the soil covered by stones for several weeks have been particularly useful as sources of predacious fungi. For example, in June 1948, by the courtesy of Dr B. Barnes, I was able to collect from a garden at Kew some specimens of plant material that had been lying for some time on the ground under blocks of paving stone. The collections consisted of stem and leaf of *Achillea millefolium* L., *Taraxacum officinale* Weber and *Oenothera biennis* L. These collections, between them, yielded *Acaulopage tetraceros* Drechsler, *Zoopage thamnospira* Drechsler, *Arthrobotrys oligospora* Fres. and an unidentified species of *Dactylella* Grove.

Dung. Since dung abounds in nematodes, it is hardly surprising that it often contains predacious fungi. Fresh horse dung is a good source of *Arthrobotrys oligospora*, and I have also isolated *A. conoides* Drechsler from horse dung supplied as garden manure. As the dung becomes more rotten, other species colonize it, and really well-decomposed dung that has been standing in a farmyard for many months may contain a very varied predacious fungus flora. A single collection from an old dung heap in a farmyard at Lowsonford, Warwickshire, which had been derelict for more than two years, yielded *Arthrobotrys oligospora*, *Stylopage hadra* Drechsler, *Cystopage lateralis* Drechsler, *Acrostalagmus obovatus* Drechsler and *Protascus subuliformis* Dang. Much work needs to be done on the distribution of predacious fungi in dung.

Living bryophytes. The possibilities of bryophytes as sources of predacious fungi seem to have been neglected by most workers in this field. Pénard (1912) observed some curious parasites in amoebae from moss, and it seems possible that he was dealing with members of the Zoopagaceae. Bryophytes, especially when moist, have a large fauna of nematodes, Protozoa and other microscopic animals, and have proved to be the most fruitful material for supplying predacious fungi that I have yet found. A small piece of moss placed in the centre of a plate of sterile maize-meal agar will often yield several different species of fungi attacking nematodes and amoebae, especially if a little soil is left attached to the rhizoids.

As an example of a particularly rich collection, the following may be quoted: A mixed collection of bryophytes was made in September 1948 from a bank bordering a lane near Hatch Bridge, Devon. The following species were present:

Brachythecium velutinum B. & S.
Dicranella heteromalla Schp.
Eurynchium praelongum B. & S.

Eurynchium myosuroides Schp.
Hypnum cupressiforme L.
Mnium hornum L.

From this collection, the following eight species of predacious fungi were obtained:

Arthrobotrys oligospora Fres.
Dactylella bembicoides Drechsler
Harposporium anguillulae Lohde
H. oxycoracum Drechsler
Nematoctonus tylosporus Drechsler

Acaulopage ischnospora Drechsler
Cochlonema sp. (probably *C. odontosperma* Drechsler)
Stylopaga hadra Drechsler

Even moss growing between paving stones in a town may contain predacious fungi. In an earlier communication (Duddington, 1950) I have described how a single small collection of *Bryum argenteum* L. from between paving stones outside a house near Kingston-on-Thames produced *Dactylella ellipsospora* (Preuss) Grove, *Dactylaria psychrophila* Drechsler, *Harposporium anguillulae* and an unidentified member of the Zoopagaceae (probably *Acaulopage* sp.) attacking amoebae. The collection was made from a point near to a gate-post where dogs were in the habit of urinating, and it is possible that an unusually rich supply of nitrogen may have been beneficial.

The reason for the rich yield of predacious fungi from moss is not clear, but two possible factors claim attention. These are the abundant supply of host animals usually present, and the high moisture content of the moss. Overgaard (1948) has published a very detailed account of the nematode population of mosses, in which he points out that the nematodes follow the movements of the water held in the moss cushions.

QUANTITATIVE ASPECTS OF HABITAT RELATIONS

As an indication of the way in which the commoner species of predacious fungi are distributed between different substrata, a list is given (Table 1) of the species found in 135 collections studied in detail from various parts of Britain. Many other collections which, for various reasons, could be investigated only superficially, have not been taken into account. From Table 1 it may be seen that the 135 collections produced 100 records of predacious fungi.

The distribution of these records among the different types of material is summarized in Table 2, and the richness of the predacious fungus flora in mosses can be clearly seen; partly decayed plant remains come second in productiveness, while leaf mould is poorest. These results must be viewed with caution, as the difficulties encountered in attempting to make a complete record of all the predacious fungi in a given sample of material are very great, and advances in technique will have to be made before this object can be achieved with certainty; moreover, the number of collections analysed in the tables is too small to form a representative sample.

Table 1. Analysis of predacious fungi in 135 collections

Type of material	Locality	Predacious fungi recorded
Leaf mould	Kingston-on-Thames, Surrey	<i>Acrostalagnus obovatus</i> Drechsler
Leaf mould	Meriden, War	None
Dung	Meriden, War	<i>Harposporium anguillulae</i> Lohde
Leaf mould	Meriden, War	<i>Acrostalagnus obovatus</i> Drechsler
Leaf mould	Solihull, War	None
Leaf mould	Solihull, War	None
Leaf mould	Solihull, War	None
Rotting wood	Guiting Wood, Glos	<i>Stylopaga haploae</i> Drechsler
Dung	Honiley, War	None
Leaf mould	Haseley, War	None
Leaf mould	Haseley, War	<i>Arthrobotrys</i> sp.
Leaf mould	Chedworth, Glos	None
Leaf mould	Chedworth, Glos	None
Leaf mould	Chedworth, Glos	None
Leaf mould	Guiting Wood, Glos	<i>Cochlonema dolichosporum</i> Drechsler
Leaf mould	Guiting Wood, Glos	None
Moss	Guiting Wood, Glos	<i>Cochlonema verrucosum</i> Drechsler
Leaf mould	Brendon, Devon	None
Leaf mould	Witcombe Wood, Glos	None
Leaf mould	Sheepscombe, Glos	None
Leaf mould	Cowley Wood, Glos	None
Leaf mould	Sheepscombe, Glos	None
Leaf mould	Fosse Bridge, Glos	<i>Dactylella cionopaga</i> Drechsler
		<i>Stylopaga hadra</i> Drechsler
		<i>Stylopaga</i> sp.
Leaf mould	Northleach, Glos	None
Rotting wood	Ravenshaw, War	None
Rotting wood	Solihull, War	None
Leaf mould	Solihull, War	None
Moss	Solihull, War	None
Moss	Stowell, Glos	None
Moss	Stowell, Glos	None
Dung	Lowsonford, War	<i>Protascus subuliformis</i> Dang.
		<i>Cystopaga lateralis</i> Drechsler
		<i>Stylopaga hadra</i> Drechsler
		<i>Arthrobotrys oligospora</i> Fres.
		<i>Acrostalagnus obovatus</i> Drechsler
Leaf mould	Wormwood Hill, Bucks	None
Leaf mould	Wormwood Hill, Bucks	None
Leaf mould	Babraham Park, Bucks	None
Leaf mould	Icknield Way, Bucks	None
Leaf mould	Copley Hill, Bucks	None
Leaf mould	Fulborne, Bucks	None
Leaf mould	Dandlebury, Bucks	None
Leaf mould	Via Devana, Bucks	None
Leaf mould	Wyre Forest, Worcs	None
Leaf mould	Wyre Forest, Worcs	None
Leaf mould	Wyre Forest, Worcs	None
Leaf mould	Wyre Forest, Worcs	None
Leaf mould	Bushwood, War	None
Leaf mould	Bushwood, War	None
Leaf mould	Shelly Green, War	<i>Arthrobotrys musiformis</i> Drechsler
Leaf mould	Barber's Coppice, War	<i>Arthrobotrys musiformis</i> Drechsler
Leaf mould	Sutton Park, Birmingham	<i>Arthrobotrys</i> sp.
Dung	Bushwood, War	None
Leaf mould	Cannon Hill Park, Birmingham	None
Leaf mould	New Forest, Hants	None
Leaf mould	Ravenshaw, War	<i>Cystopaga lateralis</i> Drechsler
Leaf mould	Aberystwyth, Cards	<i>Harposporium anguillulae</i> Lohde
Leaf mould	Chiltern Hills	None
Leaf mould	Hampton Coppice, War	<i>Arthrobotrys musiformis</i> Drechsler

Table 1 (cont.)

Leaf mould	Hampton Coppice, War	None
Leaf mould	Hampton Coppice, War	None
Leaf mould	Ambleside, Westmorland	<i>Dactylella cionopaga</i> Drechsler
Leaf mould	Bushwood, War	<i>Dactylaria</i> sp.
Leaf mould	Newton Abbott, Devon	None
Peat	Summit of Spidean Coinich, Sutherland	None
Garden compost	Chelsea, London	None
Leaf mould	Temple Balsall, War	None
Leaf mould	Temple Balsall, War	None
Dung	Lowsonford, War	<i>Harposporium anguillulae</i> Lohde
Dung	Lowsonford, War	<i>Dactylella heterospora</i> Drechsler
Dung	Lowsonford, War	<i>Meria coniospora</i> Drechsler
Dung	Lowsonford, War	<i>Arthrobotrys</i> sp.
Forest soil	Swanley, Kent	None
Leaf mould	Drayton, War	<i>Stylopaga</i> sp.
Dung	Drayton, War	None
Decaying acorns	Richmond Park, Surrey	None
Leaf mould	Richmond Park, Surrey	None
Leaf mould	Richmond Park, Surrey	None
Deer dung	Richmond Park, Surrey	None
Leaf mould	New Forest, Hants	None
Leaf mould	New Forest, Hants	None
Leaf mould	New Forest, Hants	None
Leaf mould	Esher Common, Surrey	None
Leaf mould	Lowsonford, War	None
Leaf mould	Northants	<i>Arthrobotrys oligospora</i> Fres.
Leaf mould	Oxshott Heath, Surrey	None
Leaf mould	Arbrook Common, Surrey	None
Decaying acorns	Arbrook Common, Surrey	None
Leaf mould	Littleworth Common, Surrey	None
Leaf mould	Ditton Common, Surrey	None
Leaf mould	Bishop's Stortford, Herts	<i>Arthrobotrys oligospora</i> Fres.
Artificial compost	Regent's Park, London	<i>Trichothecium flagrans</i> Duddington
Leaf mould	Netley Heath, Surrey	None
Leaf mould	Guildford, Surrey	None
Leaf mould	Ripley, Surrey	None
Leaf mould	Leith Hill, Surrey	None
Leaf mould	Abinger Hall, Surrey	None
Leaf mould	Ripley, Surrey	<i>Harposporium anguillulae</i> Lohde
Leaf mould	Oxted, Surrey	None
Garden compost	Kingston-on-Thames, Surrey	<i>Myzocyctium vermicolum</i> (Zopf) Fischer
		<i>Protascus subuliformis</i> Dang.
		<i>Stylopaga rhynchospira</i> Drechsler
		<i>Zoopage</i> sp.
		<i>Harposporium anguillulae</i> Lohde
Moss	Wimbledon Common, London	<i>Meria coniospora</i> Drechsler
		<i>Acaulopage tetraceros</i> Drechsler
		<i>Dactylella bembicoides</i> Drechsler
		<i>D. ellipsozona</i> (Preuss) Grove
		<i>Tridentaria implicans</i> Drechsler
Leaf mould	Ipswich, Suffolk	None
Leaf mould	Ipswich, Suffolk	None
Moss	Skelwith Bridge, Ches	<i>Cochlonema dolichosporum</i> Drechsler
		<i>Zoopage</i> sp.
		<i>Arthrobotrys robusta</i> Duddington sp. nov.
		ined.
		<i>Dactylella cionopaga</i> Drechsler
Decaying plant remains	Kew, Surrey	<i>Harposporium anguillulae</i> Lohde
Decaying plant remains	Kew, Surrey	<i>Zoopage thamnospira</i> Drechsler
Decaying plant remains	Kew, Surrey	None
Decaying plant remains	Kew, Surrey	None
Decaying plant remains	Kew, Surrey	<i>Arthrobotrys oligospora</i> Fres.
Decaying plant remains	Kew, Surrey	<i>Acrostalagmus obovatus</i> Drechsler
		<i>Zoopage thamnospira</i> Drechsler

Table 1 (cont.)

		<i>Acaulopage ischnospora</i> Drechsler
		<i>Cochlonema</i> sp.
		<i>Stylopage hadra</i> Drechsler
Moss	Hatch Bridge, Devon	<i>Arthrobotrys oligospora</i> Fres.
		<i>Dactylella bembicoides</i> Drechsler
		<i>Harposporium anguillulae</i> Lohde
		<i>H. oxycoracum</i> Drechsler
		<i>Nematocionus tylosporus</i> Drechsler
		None
<i>Sedum anglicum</i> Huds.	Bolt Tail, Devon	<i>Stylopage rhicnagra</i> Drechsler
Moss	Marlborough, Devon	<i>Acrostalagmus obovatus</i> Drechsler
		<i>Stylopage rhicnagra</i> Drechsler
Moss	Marlborough, Devon	<i>Dactylaria gracilis</i> Duddington
		<i>Acrostalagmus</i> sp.
		<i>Spicaria cocoospora</i> Drechsler
Moss	Wisley, Surrey	<i>Harposporium anguillulae</i> Lohde
		<i>Acaulopage baculispora</i> Drechsler
		<i>Dactylella</i> sp.
Rotting wood	Epping Forest, Essex	<i>Arthrobotrys robusta</i> Duddington sp.nov.
		ined.
Rotting wood	Wimbledon Common, London	<i>Dactylella asthenopaga</i> Drechsler
		<i>D. ellipsospora</i> (Preuss) Grove
		<i>D. asthenopaga</i> Drechsler
Decaying plant remains	Kew, Surrey	<i>Acaulopage tetraceros</i> Drechsler
		<i>Acrostalagmus obovatus</i> Drechsler
Rotting wood	Mickleham, Surrey	<i>Arthrobotrys robusta</i> Duddington sp.nov.
		ined.
		<i>Dactylella cionopaga</i> Drechsler
Moss	Mickleham, Surrey	<i>Trichothecium cystosporium</i> Duddington
Rotting plant remains	Kingston-on-Thames, Surrey	sp.nov. ined.
Rotting wood	Kingston-on-Thames, Surrey	<i>Dactylella</i> sp.
		<i>Stylopage</i> sp.
		<i>Acrostalagmus obovatus</i> Drechsler
Moss	Kingston-on-Thames, Surrey	<i>Dactylella ellipsospora</i> (Preuss) Grove
		<i>Dactylaria psychrophila</i> Drechsler
		<i>Acrostalagmus</i> sp.
Leaf mould	Wimbledon Common, London	<i>Harposporium anguillulae</i> Lohde
		<i>Harposporium anguillulae</i> Lohde
Decaying <i>Pteridium</i>	Wimbledon Common, London	<i>Dactylella bembicoides</i> Drechsler
Decaying leaves	Wimbledon Common, London	<i>Harposporium anguillulae</i> Lohde
		<i>Arthrobotrys oligospora</i> Fres.
Rotting wood	Wimbledon Common, London	<i>Harposporium anguillulae</i> Lohde
Rotting wood	Box Hill, Surrey	None
Moss	Box Hill, Surrey	<i>Dactylella bembicoides</i> Drechsler
Decaying leaves	Box Hill, Surrey	None
Moss	Box Hill, Surrey	<i>Cephalosporium balanoides</i> Drechsler
Moss	Box Hill, Surrey	<i>Dactylella cionopaga</i> Drechsler
		<i>D. bembicoides</i> Drechsler
Moss	Box Hill, Surrey	<i>Acrostalagmus</i> sp.
		<i>Nematocionus tylosporus</i> Drechsler
Moss	Box Hill, Surrey	<i>Dactylella ellipsospora</i> (Preuss) Grove
Moss	Box Hill, Surrey	None
Rotting wood	Epping Forest, Essex	<i>Harposporium anguillulae</i> Lohde
Moss	Epping Forest, Essex	None
Leaf mould	Epping Forest, Essex	<i>Dactylella asthenopaga</i> Drechsler
Moss	Epping Forest, Essex	<i>Dactylaria gracilis</i> Duddington
Dung	Origin unknown	<i>Arthrobotrys conoides</i> Drechsler

Table 2. *Distribution of records of predacious fungi among different types of material*

Type of material collected	No. of collections	No. of records
Leaf mould	76	21
Rotting wood	10	12
Partly decayed plant remains	13	17
Dung	11	11
Moss	20	39
Miscellaneous	5	0
Total	135	100

In Table 3 an analysis is made of the species of predacious fungi that occurred in the different types of material quoted in Table 1. For this purpose those records where only the genus was determined are omitted; this brings the number of records down to eighty-five. Negative information only is to be gleaned from this analysis, as the various species are fairly evenly distributed between the different kinds of material, and there

Table 3. *Distribution of species of predacious fungi among different kinds of material*

	No. of records					
	Leaf mould	Rotten wood	Partly decayed plant remains	Dung	Moss	Total
Acaulopage baculispora	.	.	.	.	1	1
A. ischnospora	.	.	.	.	1	1
A. tetraceros	.	.	1	.	1	2
Cochlonema dolichosporum	.	.	.	.	2	2
C. verrucosum	1	.	.	.	.	1
Cystopage lateralis	1	.	.	1	.	2
Stylopaga hadra	1	.	.	1	1	3
S. haploe	.	1	.	.	.	1
S. rhinacra	.	.	.	.	2	2
S. rhynchospora	.	.	1	.	.	1
Zoopage thamnospira	.	.	2	.	.	2
Arthrobotrys conoides	.	.	.	1	.	1
A. musiformis	3	.	.	.	.	3
A. oligospora	2	.	2	1	1	6
A. robusta	.	2	.	.	1	3
Dactylaria gracilis	.	.	.	.	2	2
D. psychrophila	.	.	.	.	1	1
Dactylella asthenopaga	1	2	.	.	.	3
D. bembicoides	.	.	1	.	4	5
D. cionopaga	2	1	.	.	2	5
D. ellipsospora	.	1	.	.	3	4
D. heterospora	.	.	.	1	.	1
Trichothecium cystosporium	.	1	.	.	.	1
T. flagrans	.	.	1	.	.	1
Tridentaria implicans	.	.	.	.	1	1
Acrostalagmus obovatus	2	1	2	1	1	7
Cephalosporium balanoides	.	.	.	.	1	1
Harposporium anguillulae	3	2	2	2	4	13
H. oxycoracum	.	.	.	.	1	1
Meria coniospora	.	.	1	1	.	2
Nematoctonus tylosporus	.	.	.	.	2	2
Spicaria coccospora	.	.	.	.	1	1
Myzocyttium vermicolum	.	.	1	.	.	1
Protascus subuliformis	.	.	1	1	.	2
Total	16	11	15	10	33	85

seems to be no ground for suggesting that any species is mainly confined to one particular type of habitat. *Arthrobotrys musiformis* is recorded three times from leaf mould and not from any other habitat, but this seems to be a rather uncommon species in this country; the three stations at which it was found were situated in Warwickshire within a few miles of each other, and at the time that these collections were made, leaf mould only was collected. The Zoopagaceae do not appear to differ greatly from the predacious hyphomycetes in their distribution in the table; this is in accordance with experience.

In order to gain more precise information about the distribution of predacious fungi on different kinds of substratum within a small area, a quadrat method has recently been adopted. A quadrat of suitable size (usually 1 m. square) is laid down where collections are to be taken. A list of plants growing in and around the quadrat is made, including all species that may have contributed to the litter within the quadrat, the material collected being classified into as many categories as is deemed advisable. All the samples of each particular type of material are then thoroughly mixed, so that each collection now represents a fair sample of a particular kind of material from different parts of the quadrat. The mixed samples, each of a different kind of material, are then taken back to the laboratory, and portions are planted out on maize-meal agar. The purpose of the metre quadrat is merely to focus the attention of the collector on one small area, in order to ensure that the various samples collected shall have all come from the same place, and so be strictly comparable.

This technique has not been worked long enough for any estimate to be made of its usefulness, and it is certain that a large number of quadrats will have to be examined before statistically significant results can be expected. One obvious difficulty is to ensure that the collections from within the quadrat are really random samples; since the collector is looking for particular kinds of material—decaying leaves, twigs, etc.—it is clear that the personal factor is likely to intrude. Details of four quadrats are given below as examples which were worked to test the practicability of the technique.

Quadrat no. 1

Wimbledon Common, near Wimbledon Golf Club. In a clump of birches (*Betula pubescens* Ehrh.), near a wet ditch. Surroundings wet. May 1949.

Vegetation in region of quadrat

<i>Betula pubescens</i> Ehrh.	d.	<i>Holcus lanatus</i> L.	a.
<i>Pteridium aquilinum</i> (L.) Kühn	a.	<i>Rubus fruticosus</i> agg.	o.

Material collected	Predacious fungi recorded
(1) Leaf mould. Mostly <i>Betula pubescens</i> , with some <i>Pteridium</i> litter and remains of <i>Holcus</i> . Small quantity of <i>Rubus</i> leaves probably present	None
(2) Ligneous material, mostly derived from <i>Pteridium</i> rachis with some twigs of <i>Betula</i>	{ <i>Arthrobotrys oligospora</i> Fres. <i>Dactylella bembicoides</i> Drechsler. <i>Harposporium anguillulae</i> Lohde <i>Arthrobotrys oligospora</i> Fres. <i>Cochlonema dolichosporum</i> Drechsler <i>Harposporium anguillulae</i> Lohde
(3) Soft material. Mostly dead and partly decayed leaves of <i>Betula</i>	
(4) Decaying twigs of <i>Betula pubescens</i>	

Quadrat no. 2

Box Hill, Surrey, above Juniper Hall. Small birch wood (*Betula pubescens* Ehrh.). Conditions fairly moist. Reaction of litter, pH 5.5; reaction of underlying soil, pH 4.5-5. October 1949.

Vegetation in region of quadrat

<i>Betula pubescens</i> Ehrh.	d.	<i>Pteridium aquilinum</i> (L.) Kühn	a.
<i>Rubus fruticosus</i> agg.	f.	<i>Polytrichum formosum</i> Hedw.	a.
<i>Sorbus aucuparia</i> L.	o.	<i>Eurynchium praelongum</i> B. & S.	f.
<i>Buxus sempervirens</i> L.	l.a.	<i>Thuidium tamariscinum</i> B. & S.	f.
<i>Oxalis acetosella</i> L.	o.		

Material collected

- (1) Ligneous material, mostly bark and twigs of *Betula pubescens*
- (2) Moss. *Polytrichum formosum*
- (3) Decaying leaves, mostly *Betula pubescens*
- (4) Moss. *Eurynchium praelongum* and *Thuidium tamariscinum*

Predacious fungi recorded

- Dactylella ellipsospora* (Preuss) Grove
- Dactylella bembicoides* Drechsler
- Acaulopage* sp. (Probably *A. ischnospora* Drechsler)
- Cephalosporium balanoides* Drechsler

Quadrat no. 3

North slope of Box Hill, above Juniper Hall. Chalk grassland, much grazed by rabbits. Soil pH 5.5-6.0. October 1949.

Vegetation within quadrat

<i>Poterium sanguisorba</i> L.	d.	<i>Linum catharticum</i> L.	r.
<i>Luzula campestris</i> (L.) DC.	o.	<i>Prunella vulgaris</i> L.	o.
<i>Teucrium scorodonia</i> L.	o.	<i>Holcus lanatus</i> L.	f.
<i>Potentilla reptans</i> L. var. <i>microphylla</i> Tratt.		<i>Campanula glomerata</i> L.	r.
<i>Bellis perennis</i> L.	f.	<i>Festuca</i> sp.	f.
<i>Viola riviniana</i> Rchb.	o.	<i>Sedum acre</i> L.	r.
<i>Poa annua</i> L.	f.	<i>Polytrichum formosum</i> Hedw.	f.
<i>Crepis capillaris</i> (L.) Wallr.	f.	<i>Dicranum scoparium</i> Hedw.	f.
<i>Nepeta hederacea</i> L.	o.	<i>Bryum roseum</i> Schreb.	f.
<i>Hieracium pilosella</i> L.	f.	<i>Brachythecium purum</i> Dixon	a.
<i>Lotus corniculatus</i> L.	o.	<i>Hypnum cupressiforme</i> L. var. <i>tectorum</i> Brid.	f.
<i>Thymus serpyllum</i> L.	a.	<i>Cladonia</i> sp.	o.
<i>Ranunculus repens</i> L.	f.		
	o.		

Material collected

- (1) Moss. *Bryum roseum*
- (2) Moss. *Dicranum scoparium*
- (3) Moss. *Hypnum cupressiforme* var. *tectorum* and a little *Brachythecium purum*
- (4) Moss. *Brachythecium purum*

Predacious fungi recorded

- Dactylella cionopaga* Drechsler
- Dactylella ellipsospora* (Preuss) Grove
- D. bembicoides* Drechsler
- Acrostalagmus* sp.
- Nematoclonus tylosporus* Drechsler
- Dactylella ellipsospora* (Preuss) Grove
- Acrostalagmus* sp.

Quadrat no. 4

Epping Forest, Essex, near Theydon Bois. Quadrat laid down to include a rotten stump of *Fagus sylvatica* L., of diameter 45 cm. The stump was well rotted and overgrown with mosses. Conditions moist. November 1949.

Vegetation in region of quadrat

Fagus sylvatica L.	d.	Deschampsia flexuosa (L.) Trin.	a.
Rubus fruticosus agg. (L.)	f.		

On stump:

Campylopus flexuosus Brid.	a.	Tetraphys pellucida Hedw.	a.
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Material collected

- (1) Rotten wood from beech stump
- (2) Mosses from stump
- (3) Leaf mould

Predacious fungi recorded

- { *Dactylella bembicoides* Drechsler
- { *Harposporium anguillulae* Lohde
- { *Harposporium anguillulae* Lohde
- { *Dactylella bembicoides* Drechsler
- { *D. asthenopaga* Drechsler

CONCLUSIONS

An investigation of the occurrence of predacious fungi in Britain shows that they are both abundant and widespread, and there seems no reason to believe that they are less common in this country than in the United States where, owing to the work of Drechsler, most of the records have up to now been made.

In the course of this work, representatives of nearly all the important genera have been encountered. Important exceptions are the genera *Bdellospora* Drechsler and *Endocochlus* Drechsler among the Zoopagaceae, both of which are as yet unrecorded here.

Studies in the distribution of the predacious fungi show that some species are ubiquitous, while others are less common, and much work remains to be done before any generalizations can be made concerning their habitat relations.

I should like to record my thanks to Mrs M. Peach for assistance in determining some of the angiosperm species recorded in Quadrat 3, and to Mr F. Rose and Mr F. R. Browning for their help in identifying mosses. I should like also particularly to thank Dr B. Barnes for his very valuable advice and help throughout the work.

The material here set out has formed part of a thesis for the Ph.D. degree in the University of London.

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ALBIDIN, AN ANTIBIOTIC RED PIGMENT FROM *PENICILLIUM ALBIDUM*

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Penicillium albidum Sopp grows well on all media tested and usually gives optimum antifungal titres in 4–8 days. Good titres are obtained on Czapek-Dox, Raulin-Thom and Weindling media, but not on peptone/Lemco/glucose; good titres are also obtained on a simplified medium with ammonium nitrate, potassium sulphate. When glucose concentration and depth of medium are varied, best growth per unit weight of sugar is obtained at low sugar concentrations. In general, initial pH has little effect on the activity produced.

The antibiotic albidin is obtained from Raulin-Thom culture filtrates by extraction with chloroform in yields of about 8 mg./l. In aqueous solution at pH 3 albidin loses about 90 % of its activity in 24 hr. at 25° C. and is much less stable at a higher pH. It inhibits germination of a range of moulds at concentrations varying from 0.04 to 3 µg./ml., and is bacteriostatic at concentrations of the order of 25 µg./ml. Albidin prevents oxygen uptake by *Saccharomyces cerevisiae* at 10⁻³ M, and reduces it by 82 % at 10⁻⁴ M.

A second, colourless, fungistatic substance melting at 156° C., and giving a deep green colour with ferric chloride, has been obtained. This is active against *Botrytis allii* at 25 µg./ml.

INTRODUCTION

In a preliminary publication a mould of the *Penicillium nigricans* series was shown to produce a highly fungistatic red pigment (Curtis & Grove, 1947). This mould is now considered to be *P. albidum* Sopp, and we propose to call the compound albidin. This communication is concerned with the cultural conditions for albidin production, its biological properties, and an investigation of other antifungal substances in the culture filtrates.

MATERIALS AND METHODS

Culture on liquid media

Three sizes of culture vessel were used: (a) 30 ml. lots of medium in 100 ml. conical flasks. (b) 'Glaxo' culture vessels (Clayton, Hems, Robinson, Andrews & Hunwicke, 1944), each with 250 ml. of medium unless otherwise stated. (c) Earthenware culture vessels (Abraham, Chain, Fletcher, Florey, Gardner, Heatley & Jennings, 1941), each with 500 ml. of medium.

Crude glucose was used in all the liquid media except where otherwise stated. All cultures were incubated at 25° C. The methods of sampling for assay have been described previously (Brian, Curtis & Hemming, 1947).

Assay of antifungal activity

This was done by the *Botrytis allii* spore germination test (Brian & Hemming, 1945). Titres are expressed in arbitrary B.A. units.

The mould

Several isolates of a mould falling in the *Penicillium nigricans* series were obtained from Wareham Heath soil in 1944 (Brian, Hemming & McGowan, 1945). One of these identical isolates, no. 248 in our culture collection, has been used exclusively in the work now described. On Czapek agar the colonies are floccose, sporulating sparsely, being white in colour with a greenish tinge which quickly becomes brown. The conidia are globose, very rough, $3.0-3.8\mu$, borne mainly on single sterigmata or on monoverticillate penicilli with 2-4 sterigmata; divaricate penicilli are seen infrequently. The reverse on Czapek agar is purplish brown and the colonies have a strong odour. We consider that this form approximates to *P. albidum* Sopp in the sense of Raper & Thom (1949).

RESULTS

Antagonism in agar culture

P. albidum was tested by the streak test (Brian & Hemming, 1947) for antagonism to the fungus *Candida albicans*, and the following bacteria: *Staphylococcus aureus*, *Salmonella typhi* and *Bacterium coli*. These were all inhibited, suggesting the production of a generally toxic substance.

Relationship between composition of liquid media and the development of fungistatic activity

General

Optimum titres were obtained in 4-8 days, after which the activity fell. High titres were usually associated with a pH between 3 and 5.

Preliminary results

In 100 ml. flasks good titres were obtained on Raulin-Thom, Czapek-Dox and Weindling media but not on peptone/Lemco/glucose (Table 1). In another experiment Czapek-Dox and Raulin-Thom media, both with

Table 1. *The development of fungistatic activity by Penicillium albidum on various media*

Medium	Days incubation			
	6	8	11	13
	B.A. units/ml.			
Weindling	32	4	4	2
Czapek-Dox	32	8	16	6
Raulin-Thom	64	16	8	4
Peptone/Lemco/glucose	—	—	—	—

Table 2. *The relation between pH drift and the development of antifungal activity*

	Days incubation				
	3	4	5	7	10
Raulin-Thom: pH	3.4	2.9	2.8	7.8	8.0
B.A. units/ml.	—	16	12	—	—
Czapek-Dox: pH	6.0	7.1	6.2	4.9	6.1
B.A. units/ml.	—	—	—	32	8

2.5 % crude glucose, were used in earthenware culture vessels (Table 2). At first the pH of the Raulin-Thom fell and that of the Czapek-Dox rose. This was probably due to the utilization of ammonium and nitrate nitrogen respectively. After 4 days the pH of the Czapek-Dox also fell. The peak antifungal titre occurred in both cases when the pH was at a minimum, before a final rise.

Effect of glucose concentration and depth of medium on antibiotic production

Raulin-Thom medium with 2.5, 5 and 10 % glucose was used with three different volumes of medium in Glaxo vessels, i.e. 125, 250 and 500 ml. (Table 3). High glucose concentrations kept the pH lower, but did not alter the peak titre, though the incubation period was increased. Large volumes of medium also delayed the peak and gave lower titres. Mycelial weight increased with both sugar concentration and volume of medium, but, for an equal weight of sugar supplied, low concentrations gave more mycelium than high concentrations.

pH and nitrogen source

In earlier work replacement of nitrate in a simplified medium by other nitrogen sources had a considerable effect on the metabolism of *Myrothecium verrucaria* (= *Metarrhizium glutinosum*) (Brian *et al.* 1947) and *Penicillium gladioli* (Brian *et al.* 1948). With *P. albidum* similar experiments in Glaxo vessels were carried out with potassium nitrate (N), ammonium nitrate (AN), ammonium sulphate (AS) and ammonium tartrate (A) media with 5 % glucose, each adjusted to different pH values from 3 to 6 (Table 4). *P. albidum* grew well on all these media. In this respect it was rather similar to *P. gladioli* but differed from *Myrothecium verrucaria*, as the latter grew poorly on media AS and AN. In general initial pH appeared to have little effect on the metabolism and subsequent pH drift. *Penicillium albidum* resembled *P. gladioli* in giving good titres on nitrate media (N and AN). With medium AN the pH rapidly changed to about 3.5, and after this the activity slowly decreased. Medium N was exceptional in that good titres occurred at pH 7, but activity was then lost rapidly. At this pH albidin is extremely unstable and the activity may have been due to another substance (see below). With medium A adjusted to pH 3-4, the pH fell rapidly to about 2.5 and remained at this level. With a higher initial pH it fell to pH 3 and then rose to about pH 6.5. *P. albidum* resembled *Myrothecium verrucaria* and *Penicillium gladioli* in giving good titres on this medium, but poor on medium AS. With the latter, utilization of ammonium ions rapidly produces a very low pH which may be inimical to antibiotic production.

Extraction of albidin

For the production of albidin, Raulin-Thom medium with 2.5 % glucose was used in earthenware culture vessels. After incubating for 5 days, culture filtrates were extracted with chloroform, the chloroform was evaporated to dryness and the residue crystallized from ethanol, giving yields of about 8 mg./l. of albidin. Results from typical batches are shown

Table 3. *Effect of glucose concentration and volume of medium/culture vessel on the development of antifungal activity*

% glucose	ml./Glaxo vessel	Days growth												Mean final dry weight of mycelium (g./flask)	
		B.A. units/ml.						pH							
		4	7	10	14	18	22	0	4	7	10	14	18		22
2.5	125	24	—	—	—	—	—	4.4	3.3	8.1	8.4	7.9	8.1	7.9	0.6
5.0	125	32	32	—	—	—	—	4.2	3.1	3.0	8.0	7.2	6.6	7.9	1.0
10.0	125	6	24	8	4	4	2	4.3	3.2	2.9	3.0	3.3	3.6	4.2	1.7
2.5	250	8	—	—	—	—	—	4.4	3.4	6.8	8.3	8.1	8.2	7.7	1.4
5.0	250	4	16	8	—	—	—	4.2	3.2	2.9	8.3	6.5	5.0	7.6	2.3
10.0	250	2	16	12	4	4	4	4.3	3.3	2.8	3.0	3.0	3.3	2.9	3.4
2.5	500	—	8	2	—	—	—	4.4	3.9	3.8	6.7	7.9	8.1	7.2	4.8
5.0	500	—	4	6	4	—	—	4.2	3.7	3.0	3.1	3.8	6.3	6.5	5.0
10.0	500	—	4	12	8	8	4	4.3	3.7	3.0	3.0	3.2	3.7	2.7	6.0

Table 4. *The effect of pH and nitrogen source on the development of fungistatic activity*

	Initial pH	Days growth										Mean final dry weight of mycelium (g./flask)		
		B.A. units/ml.					pH							
		5	9	13	17	20	5	9	13	17	20			
medium AN, ammonium nitrate	3.1	32	24	8	4	2	3.2	3.1	3.1	3.3	3.2	1.7		
	4.0	32	16	12	4	4	3.4	3.3	3.2	3.2	3.3	1.6		
	4.5	32	24	16	4	2	3.5	3.3	3.3	3.2	3.4	1.6		
	5.4	32	24	16	4	2	3.8	3.5	3.5	3.4	3.5	1.6		
N, potassium nitrate	3.2	32	4	8	—	—	7.1	5.4	5.7	6.3	7.2	1.9		
	4.0	16	8	12	—	—	7.4	5.3	5.5	6.3	7.2	1.9		
	4.5	8	16	12	2	—	7.3	5.2	5.1	6.1	7.1	1.9		
	5.7	8	8	8	2	—	7.2	5.5	5.3	6.0	7.1	1.8		
AS, ammonium sulphate	3.9	—	—	4	—	—	1.9	1.8	1.9	2.1	1.9	2.1		
	4.1	—	—	—	—	—	1.9	1.8	1.8	2.0	1.9	2.0		
	4.6	—	—	2	—	—	1.9	1.9	1.9	1.9	2.0	2.1		
	5.5	—	—	2	—	—	1.9	1.9	1.9	1.9	2.0	2.1		
Days growth														
		Days growth										Mean final dry weight of mycelium (g./flask)		
		B.A. units/ml.					pH							
		4	7	10	14	17	20	4	7	10	14		17	20
A, ammonium tartrate	3.0	16	—	—	—	—	2	2.0	1.8	1.9	1.9	1.8	1.9	2.0
	3.9	32	16	—	—	—	2	2.3	2.3	2.3	2.4	2.5	2.8	1.7
	4.9	16	16	4	2	—	—	2.8	2.6	2.7	3.6	5.8	6.1	1.9
	6.0	16	16	8	—	—	—	3.1	2.9	2.9	3.7	6.1	6.8	1.9

Albidin, an antibiotic red pigment from Penicillium albidum 337

in Table 5. A brief characterization of this compound has already been published (Curtis & Grove, 1947).

Subsequent experiments showed that benzene, butanol, carbon tetrachloride and petrol ether were not as effective as chloroform for extracting albidin.

Table 5. *The production of albidin*

Batch no.	Vol. of culture filtrate (l.)	pH of culture filtrate	B.A. units/ml. culture filtrate	Yield (mg./l.)
374	39	4.1	16	7.9
382	30	3.6	16	
383	40	3.5	16	
384	33	3.6	16	
387	41	3.8	24	
388	44	3.4	16	9.0
392	44	3.5	16	
393	38	5.3	8	
396	40	4.0	16	7.7
401	64	3.6	16	
402	64	3.7	32	
403	40	3.5	16	
404	64	3.5	16	
405	40	3.4	16	7.8
407	42	3.0	16	
408	43	3.3	16	

Stability of albidin

Aqueous solutions from pH 3 to 8 were kept at 25° C. and assayed after 24 hr. The activity which remained was as follows:

pH	3.3	4.0	5.1	6.4	7.1	7.9
Remaining activity (%)	12.5	9.4	4.7	<2.0	<2.0	<2.0

Albidin is very unstable over the whole range, but it is more stable at pH 3 than at a higher pH. As the antibiotic decomposed the orange solutions if alkaline changed to brown, and if acid first became colourless, then pale brown.

Biological activity of albidin

Fungistatic tests

The minimum concentration to inhibit a range of moulds in Weindling medium varied from 0.04 to 3 µg./ml. (Table 6). Corresponding figures for glutinosin for a similar though not identical range are 0.2–25 µg./ml. (Brian *et al.* 1947), and for viridin 0.02–50 µg./ml. On this basis albidin is more active than glutinosin and differs from viridin in being less selective.

Antibacterial tests

In nutrient broth at pH 7 albidin inhibits *Staphylococcus aureus* at 50 µg./ml. and *Salmonella typhi* at 25 µg./ml.

Effect of albidin on oxygen uptake by Saccharomyces cerevisiae in the presence of glucose

Washed baker's yeast cells were suspended in McIlvaine buffer (pH 3.5) containing 1 % (w/v) glucose (AR), at a concentration of 1 % fresh-weight/

volume. The rate of oxygen uptake at 25° C. was measured in a Barcroft respirometer in the presence of albidin at concentrations of 10^{-3} , 10^{-4} and 10^{-5} M, calculated on the basis of a molecular formula $C_{10}H_8O_4$. At the two lower concentrations the initially pale red solutions were almost immediately decolorized, and in the highest concentration (10^{-3} M) the intensity of the colour was markedly reduced. At 10^{-3} M albidin completely inhibited oxygen uptake, at 10^{-4} M it reduced oxygen uptake to 18 % of the rate in controls, but at 10^{-5} M it was without effect. Albidin is therefore a moderately effective inhibitor of glycolysis.

Table 6. *Fungistatic activity of albidin. Least concentration (μ g. ml.) preventing germination in Weindling medium (pH 3.5)*

<i>Absidia glauca</i> Hagem	1.6
<i>Aspergillus niger</i> v. Tiegh.	1.6
<i>A. terreus</i> Thom	0.8
<i>Botrytis allii</i> Munn.	0.2
<i>Fusarium graminearum</i> Schwabe	0.04
<i>F. caeruleum</i> (Lib.) Sacc.	0.4
<i>Mucor mucedo</i> Bref.	0.4
<i>Myrothecium verrucaria</i> (Alb. & Schw.) Ditmar	1.6
<i>Penicillium brevicompactum</i> Dierckx	0.4
<i>P. digitatum</i> Sacc.	0.8
<i>P. expansum</i> Link	1.6
<i>P. gladioli</i> McCull. & Thom	0.4
<i>Stachybotrys atra</i> Corda	3.1
<i>Stemphylium</i> sp.	3.1
<i>Thamnidium elegans</i> Link	0.04
<i>Trichoderma viride</i> Pers. ex Fries	3.1
<i>Verticillium cinnabarinum</i> (Cda.) Reinke & Berth.	3.1

A SECOND ANTIFUNGAL SUBSTANCE PRODUCED BY *PENICILLIUM ALBIDUM*

After two extractions with one-tenth volume of chloroform about 25 % of the antifungal activity remained in Raulin-Thom culture filtrates. This is far in excess of that which remains when albidin solutions are extracted. The residual activity is not extracted by chloroform, *n*-butanol or ethyl acetate. Weindling batches with similar titres to Raulin-Thom yield little albidin.

The activity in Weindling batches was adsorbed by 2 g. l. of charcoal (B.D.H.), and eluted with chloroform. The residue from this eluate was extracted with cold ether. On concentrating this extract pale brownish needle-shaped crystals separated. This solid, after several recrystallizations from benzene, gave colourless crystals melting at 156° C. with decomposition. It contained no nitrogen, sulphur or halogen, and left no ash on combustion. It gave a deep green colour with ferric chloride in ethanol, and was active at 25 μ g./ml. against *Botrytis allii*. The yields were about 4 mg./l., accounting for only 0.5 % of the activity in the culture filtrate, but further work did not lead to better yields or the isolation of other fungistatic substances.

We wish to thank Dr P. W. Brian for much encouragement and advice; we are also indebted to him for identification of the mould which produces albidin and for the data on oxygen uptake. We are indebted to Mr J. F.

Albidin, an antibiotic red pigment from Penicillium albidum 339

Grove for assistance with the characterization of the colourless antibiotic. We also wish to thank Mr D. Scott for assistance in the production of albidin.

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A SURVEY OF HETEROTHALLISM IN THE USTILAGINALES

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The existence of heterothallism has been established in a total of about thirty species of Ustilaginales, through the work of numerous authors. In the majority of these species, the heterothallism has been found to be of a simple type, with two strains, + and -, but in a few species claims have been made for the existence of more complex types of heterothallism. Critical study has shown that these claims are in every instance based on faulty reasoning and cannot be substantiated. A negative result for a test for mating-type need not necessarily imply that the pair of cultures investigated are sexually incompatible, since genetic or environmental factors causing weak pathogenicity, or preventing conjugation of spores on agar, or preventing the subsequent development of aerial mycelium, may invalidate the test. Claims for the existence of a second locus controlling heterothallism are shown to be due to genetic factors having such effects. The supposed multiple allelomorphs controlling heterothallism in *Ustilago longissima* are evidently factors affecting the appearance of the hyphae in culture, or affecting the pathogenicity of the fungus, and are not factors for heterothallism, while the three self-incompatible but mutually cross-compatible strains are readily explained if the third strain was a sterile dikaryon. It is concluded that heterothallism in all the species of Ustilaginales that have so far been investigated is controlled by a single pair of allelomorphs.

INTRODUCTION

Heterothallism in the Ustilaginales was first discovered by Kniep (1919) in *Ustilago violacea*, where he found two strains, + and -, which occurred with approximately equal frequencies. Since then about thirty species of smut fungi have been found to be heterothallic—in fact, all those so far examined in detail. *Grahiola phoenicis* Poit., studied by Killian (1924), and *Urocystis cepulae* Frost, investigated by Blizzard (1926), would appear to be homothallic, but no conclusive tests have been made. Cytological studies of *Cintractia montagnei* (Tul.) Magn. by Rawitscher (1922) suggest that this species may be secondarily homothallic, with + and - nuclei included in each basidiospore at the time of its formation, but this does not appear to have been confirmed experimentally. *Ustilago ischaemi* Fuckel, studied by Boss (1927), appears to be apomictic, with no nuclear fusion in the life cycle. With most of the heterothallic species of Ustilaginales, there is clear evidence for the existence of only two strains, + and -, within each species, but in a few instances claims have been made for the existence of more complex types of heterothallism, such as are known to occur in the Hymenomycetes and Gasteromycetes. The evidence on which these claims are based has been examined in an attempt to clarify the present position, and the results are presented below.

METHODS OF DETERMINING MATING-TYPE

Two basically different methods have been used for determining the mating-types of basidiospores of smut fungi. These two methods are as follows:

(a) *Method dependent on the behaviour or appearance of the fungus in culture when two single spores (or the mycelium derived from them) are paired.* When two basidiospores germinate in proximity on nutrient agar, if the hyphae fuse the spores are considered to be of different and compatible mating-types; if the spores fail to conjugate they are considered to be of the same or at least of incompatible mating-types. Bauch (1922) found that in *U. violacea*, mating-types could be determined from the general appearance of the paired cultures. When the two cultures were of different mating-types, considerable aerial mycelium was produced, whereas when of the same mating-type there was little or no aerial growth. This technique has since been applied to a number of species (Bauch, 1927, 1932*a*, *b*; Tyler, 1934; Rodenhiser, 1934).

(b) *Method dependent upon the behaviour or appearance of the fungus when paired cultures are inoculated into the host plant.* This method of determining mating-types has been used chiefly with species in which the first method cannot be used because the spores fail to conjugate on agar even when they are of compatible mating-types. It appears to be well established that extensive infection of host plants by heterothallic species of Ustilaginales is dependent on the presence of dikaryotic mycelium containing nuclei of two different mating-types, and that single-spore inoculations normally fail to cause infection. Consequently, if infection occurs after inoculation by a pair of cultures, it is assumed that the two strains are of compatible mating-types, and if infection does not occur that they are of incompatible mating-types. In certain instances, the pigmented appearance of the host near the point of inoculation or the general chlorotic appearance of the host plant (Tyler, 1934, 1938) when infection is occurring, serves as a rapid test of mating-type, without the need to wait for the appearance of chlamydospores as an indication of successful infection of the host.

Both these methods of determining mating-type have certain disadvantages. With the spore-conjugation method (or Bauch's modification of it, using colony appearance), the occurrence of conjugation or the development of aerial hyphae has been shown to depend to some extent on the cultural conditions. Thus Hanna (1934) showed that conjugation of basidiospores in *Tilletia caries* [*T. tritici*] was greatly influenced by the acidity of the medium, and Holton (1932) and Bowman (1946) found a marked influence of the nutritive value of the culture medium and of the incubation temperature. Bauch (1932*a*, *b*), Fischer (1940*a*) and Holton and Fischer (1941) found variation in behaviour after conjugation of compatible basidiospores in various species and species crosses. Binucleate aerial infection hyphae ('Suchfaden' of Bauch) were sometimes formed, while in other instances only a tangle of fine threads ('Wirrfaden' of Bauch) developed on the surface of the culture medium.

With the host-infection method, Rodenhiser (1934) has pointed out that

'the fact that smut is not produced in the host plant as a result of inoculation with a given mating does not necessarily mean that the lines are of the same sex, for in some matings incompatibility may be due to the presence of sterility factors'. In particular, it is clear that if one of the paired cultures carries a dominant gene for weak pathogenicity, or if both carry a recessive gene producing such an effect, then it is likely that the host will not be infected, even though the two cultures are sexually compatible. Thus, Holton and Fischer (1941) found that in crosses between single-spore cultures of *Ustilago perennans* and *U. kollerii*, *U. avenae* and *U. hordei*, after basidiospores had conjugated on agar, an infection hypha sometimes failed to form, and in just these instances no infection of the host plant occurred when the paired cultures were inoculated. It is evident that these spores, though compatible as shown by conjugation, were yet incapable of infecting the host. The conclusion that two spores or cultures are incompatible, because they fail to produce smut in the host when inoculated together, is clearly erroneous. When this error is made, a gene for weak pathogenicity may resemble a second locus for heterothallism. Similar arguments can be applied to the spore-conjugation method (or its modification). A gene which limits the occurrence of conjugation to particular environmental conditions, or suppresses the development of aerial mycelium in compatible cultures, would thus resemble in its effects a second locus controlling heterothallism, unless it be accepted that a negative result for a mating-type test does not necessarily imply that the spores or cultures are sexually incompatible.

THE CHARACTERISTICS OF THE KNOWN TYPES OF HETEROTHALLISM

(a) *Two-allelomorph compared with multiple-allelomorph heterothallism*

Heterothallism in the Mucorales and in the Ascomycetes (except the Laboulbeniales) appears to be determined by a pair of allelomorphs, whereas in the Hymenomycetes and Gasteromycetes it appears to be determined by multiple allelomorphs. In order to distinguish between two-allelomorph and multiple-allelomorph heterothallism, it is essential to obtain material of the fungus from more than one source, since any one fruit-body normally contains only two allelomorphs at a given locus on a chromosome. The existence of multiple allelomorphs is suspected if all pairings between spores from different fruit-bodies are compatible. Proof of the existence of multiple allelomorphs requires in addition the demonstration that there is no recombination in the next generation between the factors controlling heterothallism present in the material from the two different sources. Thus, if one source of the fungus supplies strains + and -, and a second source supplies + ' and - ', then, if the factors for heterothallism from the first source are allelomorphic with those from the second, when say a + culture is crossed with a + ', the strains will be found to be compatible and, moreover, these two types, and no others in addition, will be produced in the next generation. If, on the other hand, the factors are non-allelomorphic, two new mating-types, in addition to the parental ones, would be expected.

In the Ustilaginales, it is likely that in many instances the infection of

individual host plants results from one original pair of basidiospores. If so, all the chlamydospores on one host individual would carry the same pair of allelomorphs, even though different hosts might carry different allelomorphs. Hence, in general, the demonstration of the existence of multiple allelomorphs controlling heterothallism in a smut fungus is likely to require the pairing of single basidiospores from one host individual with single basidiospores from another host individual. The existence of compatibility in all such pairings would suggest the occurrence of multiple-allelomorph heterothallism. Conversely, a correspondence in mating-type between the strains from one individual and those from another would suggest two-allelomorph heterothallism. If correspondence is found between + and - strains when the host individuals are from widely-separated localities, then the existence of only two allelomorphs controlling heterothallism is virtually proved. Furthermore, where it has been found possible to hybridize related species of smut fungi and to demonstrate that the + and - strains correspond in the two species, there can be no doubt that heterothallism is two-allelomorphic.

(b) Bipolar compared with tetrapolar heterothallism

When spores of only two mating-types are found on a fungal fruit-body, the heterothallism is described as bipolar. When spores of four different mating-types are found, such that type A is compatible with B, and C with D, but all other combinations are incompatible, then the heterothallism is described as tetrapolar. The bipolar condition implies that heterothallism is determined by allelomorphs at one locus, the tetrapolar condition by allelomorphs at two loci. Tetrapolar heterothallism is found in many of the Hymenomycetes and Gasteromycetes, where multiple allelomorphs exist at the loci concerned. It has been shown that the tetrapolar condition is essentially an outbreeding device (Mather, 1942), and that it is effective as such only when associated with multiple allelomorphs (Whitehouse, 1949). On theoretical grounds it therefore appears unlikely that the tetrapolar condition will be found associated with two-allelomorph heterothallism.

In the Ustilaginales, the bipolar or tetrapolar condition can be determined by pairing the basidiospores from a single chlamydospore with one another, or by pairing basidiospores chosen at random from amongst the chlamydospores from a given host individual (assuming all the chlamydospores on one host individual carry the same pair of allelomorphs at a given locus). If multiple allelomorphs are absent, the pairing of basidiospores from any source, whether from the same or different host individuals, will show whether two or four mating-types are present.

EXPERIMENTAL RESULTS

As already stated, in a majority of the species of Ustilaginales that have been found to be heterothallic, there is clear evidence that the heterothallism is of a simple type, with + and - strains controlled by a single pair of allelomorphs. The species in which two-allelomorph heterothallism has been found are listed in Table 1. References to the experimental work

are given for each species, in all of which the mating-types have been recognized by the spore-conjugation method (or Bauch's modification). In some instances, confirmation has been obtained by the host-infection method. Thus Holton (1931) found that with *U. avenae* and *U. kolleri* 'all combinations of monosporidial lines in which fusions were observed in culture produced chlamydospores on the host' and 'no smut was produced by inoculation... with combinations of monosporidial lines which did not

Table 1. *Species of Ustilaginales in which heterothallism determined by one pair of allelomorphs has been found*

Species	References
<i>Ustilago violacea</i> (Pers.) Fuckel	Kniep, 1919; Zillig, 1921; Bauch, 1922; Goldschmidt, 1928
<i>U. scabiosae</i> (Sow.) Wint.	Kniep, 1921
<i>U. bromivora</i> (Tul.) Fisch. v. Waldh.*	Bauch, 1925
<i>U. grandis</i> Fr.	Kniep, 1926
<i>U. anomala</i> J. Kunze	
<i>U. cardui</i> Fisch. v. Waldh.	
<i>U. tragopogonis-pratensis</i> (Pers.) Roussel	
<i>U. tritici</i> (Pers.) Rostr.*	
<i>U. utriculosa</i> (Nees) Tul.	
<i>U. vinosa</i> Tul.	
<i>U. hordei</i> (Pers.) Lagerh.	Kniep, 1926; Dickinson, 1927
<i>U. nuda</i> (Jens.) Rostr.*	Kniep, 1926; Thren, 1937; Tapke, 1943
<i>U. perennans</i> Rostr.*	Kniep, 1926; Holton & Fischer, 1941
<i>U. kolleri</i> Wille [syn. <i>U. levis</i> (Kellerm. & Swingle) Magn.]*	Dickinson, 1927, 1931; Hanna & Popp, 1930; Holton, 1931; Popp & Hanna, 1935
<i>U. avenae</i> (Pers.) Rostr.	Kniep, 1928; Hanna & Popp, 1930; Holton, 1931; Holton & Fischer, 1941
<i>U. succisae</i> Magn.	Kniep, 1928
<i>U. scorzonerae</i> Schroet.	Bauch, 1932 <i>b</i>
<i>U. nigra</i> Tapke*	Allison, 1935, 1937 (as <i>U. medians</i> Bied.); Bever, 1945
<i>U. striiformis</i> (Westd.) Niessl	Fischer, 1940 <i>a</i>
<i>U. bullata</i> Berk.	Fischer, 1940 <i>b</i>
<i>Sphacelotheca cruenta</i> (Kühn) Potter	Rodenhiser, 1932, 1934

* According to Fischer (1943), *Ustilago perennans* and *U. nigra* are synonyms of *U. avenae*; *U. kolleri* a synonym of *U. hordei*; *U. tritici*, a synonym of *U. nuda*; and *U. bromivora*, a synonym of *U. bullata*.

Table 2. *Species of Ustilaginales in which the existence of complex types of heterothallism has been claimed*

Species	References
<i>Ustilago hypodytes</i> (Schlecht.) Fr.	Bauch, 1934
<i>U. longissima</i> (Schlecht.) Tul.	Bauch, 1923; Kniep, 1926 Kämmerling, 1929; Bauch, 1930, 1931, 1934 Stakman & Christensen, 1927; Hanna, 1929; Eddins, 1929; Christensen, 1929, 1931; Sleumer, 1932; Bauch, 1932 <i>b</i>
<i>U. longissima</i> var. <i>macrospora</i> Davies	
<i>U. maydis</i> (DC.) Corda [syn. <i>U. zeae</i> Unger.]	
<i>Sphacelotheca destruens</i> (Schlecht.) Stev. & A. G. Johns. [syn. <i>S. panicimiliacei</i> (Pers.) Bubák]	Bauch, 1934
<i>S. reiliana</i> (Kühn) Clint. [syn. <i>Sorosporium reilianum</i> (Kühn) McAlpine]	Hanna, 1929; Tyler & Shumway, 1935; Vaheeduddin, 1936
<i>S. schweinfurthiana</i> (Thüm.) Sacc.	Bauch, 1932 <i>a</i>
<i>S. sorghi</i> (Link) Clint.	Rodenhiser, 1932, 1934; Isenbeck, 1935; Tyler, 1938
<i>Tilletia caries</i> (DC.) Tul. [syn. <i>T. tritici</i> (Bjerk.) Wint.]	Flor, 1932; Hanna, 1934; Becker, 1936
<i>T. foetida</i> (Wallr.) Liro [syn. <i>T. levis</i> Kühn]	

fuse in culture'. In many instances, species crosses have shown that the mating-types correspond in different species, thus establishing beyond question that the heterothallism is of the two-allelomorphic type. Such correspondence has been found within the group of species *U. grandis*, *U. tritici*, *U. avenae*, *U. kolleri*, *U. hordei*, *U. nigra* (including *U. medians*), *U. perennans*, *U. bromivora*, *U. nuda*, *U. bullata* and *U. striiformis* by Kniep (1926), Hanna and Popp (1930), Holton (1931, 1932, 1936), Bauch (1934), Allison (1935, 1937), Fischer (1940a, b), Holton and Fischer (1941), and Bever (1945); and between *U. violacea*, *U. scabiosae*, *U. cardui*, *U. utriculosa*, *U. vinosa*, *U. anomala* and *U. tragopogonis-pratensis* by Kniep (1926).

Species of Ustilaginales in which the existence of complex types of heterothallism has been claimed are listed in Table 2, together with references to the experimental work. Each of these species will be discussed in turn.

(a) *Ustilago longissima* (Schlecht.) Tul. and *U. longissima* var. *macrospora* Davis

This species and its variety parasitize grasses of the genus *Glyceria* and have been extensively studied by Bauch and Kämmerling. In his early work, Bauch (1923) found from a study of the formation of infection hyphae after conjugation on agar, that material of *Ustilago longissima* from Vötting, near Freising, comprised strains of two mating-types, which he called A and B. Material of the var. *macrospora* also showed two mating-types, and, moreover, when species and variety were crossed it was found that the mating-types corresponded. These observations, made at the start of Bauch's work on this species, constitute virtual proof that the species and its variety possess two-allelomorph heterothallism, since it is highly improbable that the mating-types of species and variety would happen to correspond, if multiple strains existed. Bauch found that material of *U. longissima* from Krampnitzsee, near Potsdam, Berlin, also comprised two mating-types. One of these strains he considered to be different from either of those in the Vötting material, since it was compatible with both the A and B strains (spore-conjugation test), and so he described the strains of the Krampnitzsee material as A and C. Unfortunately, Bauch does not present his experimental work in sufficient detail to judge the validity of this conclusion, but in view of the correspondence in mating-type between species and variety, the existence of a third allelomorph seems unlikely. An alternative explanation, which does not require the existence of a third allelomorph, appears more likely to be correct. If in making crosses between the Krampnitzsee and Vötting material, a strain of C was used that happened to be dikaryotic and to contain both A and B nuclei, conjugation of the C strain with both the A and B strains of the Vötting material would probably result. Such dikaryotic strains might be expected not infrequently, since Bauch showed that the first basidiospore cut off from the promycelium was frequently binucleate.

Kniep (1926) crossed Bauch's three strains of *U. longissima* with *U. bullata* [*U. bromivora*], *U. hordei* and *U. perennans* and found that the mating-types of two of the three strains of *U. longissima* corresponded with the two

mating-types of the other three species, while the third strain of *U. longissima* conjugated with both strains of each of the other three species. These observations strongly support the hypothesis of two-allelomorph heterothallism in *U. longissima*, with the third strain a plus-minus dikaryon. Subsequently, Kämmerling (1929) supported Bauch's original claim for the existence of three strains A, B and C, but a dikaryotic tester strain could also explain these results.

From studies of material from two sources in Hungary and one near Rostock, Bauch (1930) claimed to have found tetrapolar heterothallism. It is evident, however, that his second locus concerned the development of 'Wirrfaden' or 'Suchfaden' subsequent to conjugation of compatible strains in culture media. His data are in agreement with the hypothesis that mating-type is controlled by one pair of allelomorphs, the type of hypha subsequently produced being controlled by other factors, which should not be considered as a second locus for heterothallism. Bauch (1930, 1931) gave a detailed account of numerous strains of *U. longissima* obtained from various localities in Europe. Many were unique in their pairing reactions, as regards the formation of 'Wirrfaden' and 'Suchfaden', and Bauch concluded that multiple factors were involved. However, as stated above, factors controlling the formation of these two types of hyphae cannot be regarded as an essential part of the mating system. There is no evidence in any of this work of Bauch's for the existence of more than one locus controlling mating-type in *U. longissima*.

Bauch (1934) published details of the results of pairing single-spore cultures of various species of smut fungi, including *U. longissima* and certain other species in which 'multipolar sexuality' was supposed to occur. Of the three strains of *U. longissima* studied (called 0, 1 and 2), it would appear that strain 0 was of one mating-type, strain 2 of the opposite mating-type and strain 1 a dikaryon containing nuclei of opposite mating-types. Strain 1 by itself failed to produce 'Suchfaden', and hence Bauch concluded that it was of a third mating-type comparable with the other two. However, it seems probable that this strain contained nuclei of genetic constitution such that 'Suchfaden' were not formed, for the earlier work has clearly shown that this property is under genetic control. Such a sterile dikaryon would closely resemble a 'third mating-type'. Almost all the results given by Bauch (1934) of pairings between different species of smut fungi are in agreement with the hypothesis that mating-types in each species are controlled by a pair of allelomorphs. Thus, it would appear from Bauch's data that the 'A' strains of *U. bullata*, *U. avenae*, *U. perennans* and *U. hordei*, the 'B' strain of *U. grandis*, the 'o' strains of *U. maydis* and *Sphacelotheca destruens*; the '1' strain of *S. schweinfurthiana* and the '2' strain of *Ustilago longissima* were all of one mating-type, while the 'B' strains of *U. bullata*, *U. avenae*, *U. perennans* and *U. hordei*, the 'A' strain of *U. grandis*, the '1' strains of *U. maydis* and *Sphacelotheca destruens* and the 'o' strains of *S. schweinfurthiana* and *Ustilago longissima* were all of the opposite mating-type. Thirty-one pairings within the first group of strains showed twenty-nine negative results and two positive. Thirty-one pairings within the second group of strains gave thirty negative results and one positive. The three positive results are clearly within the bounds of experimental error and

might be due to accidental infection by spores of the opposite mating-type. Seventy-one pairings between strains of the first group and those of the second gave forty-seven positive results and twenty-four negative. All the negative results were with interspecific pairings, where intersterility due to causes other than sexual compatibility might be expected. The fact that correspondence in mating-types can be detected between all these species provides good evidence for the existence of only two allelomorphs controlling heterothallism in all of them.

(b) *Ustilago hypodytes* (Schlecht.) Fr.

Bauch (1934) records that this species has three sexual genes, but he does not give the evidence on which this claim is based. It seems probable that alternative explanations of the observations, as suggested for *U. longissima*, are more likely to be correct.

(c) *Ustilago maydis* (DC.) Corda [syn. *U. zeae* (Beckm.) Unger]

Indications that this species is heterothallic were found by Stakman and Christensen (1927), and conclusively demonstrated by Hanna (1929). Very extensive experimental studies of heterothallism in this species have been made by Christensen (1929, 1931). Most workers with *U. maydis* have found that spore conjugation did not occur with regularity, and so have been compelled to use the more laborious host-inoculation method in testing for mating-types. Sleumer (1932), Bauch (1932*b*), and Bowman (1946) have, however, been able to use the spore-conjugation method or Bauch's modification of it.

Hanna (1929) was the first to investigate the mating-types of single basidiospores. He analysed four basidiospores from each of seven chlamydospores. Six of the chlamydospores came from St Paul, Minnesota and the seventh from Bari, Italy. When basidiospores from one chlamydospore were paired individually with basidiospores from another chlamydospore, two mating-types were demonstrated in every instance. Moreover, the mating-types corresponded in the American and Italian material. This is a clear indication that the heterothallism is determined by a single pair of allelomorphs. When the stocks were inbred by pairing basidiospores derived from the same chlamydospore, evidence for the existence of only two mating-types was again found in most instances, but with one chlamydospore (no. A1), host infection resulted when basidiospore no. 1 was paired with no. 3, and when no. 2 was paired with no. 4, but in no other pairings. Hanna interpreted this as tetrapolar heterothallism, but as indicated on p. 342 above, this deduction is not justified. With another of the chlamydospores (no. C2), when basidiospore no. 1, was paired with no. 4, and no. 2 with no. 3, only weak infection resulted. Obviously, if compatible cultures sometimes produce only weak infection it is likely that in other instances complete failure to infect the host may result, even though the cultures are of different mating-types. The fact that this failure to infect the host, when inoculated with cultures of opposite mating-type, was only made evident on inbreeding, suggests that recessive genes for weak pathogenicity were responsible. If a negative result in a mating-type test is not

necessarily taken to imply that the two cultures that were paired are incompatible, then all Hanna's data agree with the hypothesis that heterothallism in *U. maydis* is controlled by one pair of allelomorphs.

Eddins (1929) studied six single-basidiospore cultures of *U. maydis*, and from his analysis of mating-types he concluded that there were four sexual groups in this species. However, he made the same error as Hanna: if the negative reaction when his spores A and D were paired is not taken to imply that these spores are necessarily incompatible, then his data support the hypothesis of two mating-types (A and B versus C and D.) One of the basidiospores caused infection when injected alone into the host, and Eddins concluded that the species is sometimes homothallic. It seems probable that this spore was dikaryotic and contained both plus and minus nuclei.

Christensen (1929) crossed each of two single-basidiospore cultures of opposite mating-type with seventeen other single-basidiospore cultures, derived from various parts of the world. In every instance the cultures were compatible with one or other of the two tester strains, but never with both. Christensen realized that this showed the absence of multiple allelomorphs, but he failed to realize that it also showed the absence of more than two mating-types. He subsequently inbred his stocks by pairing basidiospores derived from the same chlamydospore, just as Hanna had done, and so encouraged recessive factors for weak or deficient pathogenicity to become manifest. The host-inoculation method was thus rendered unreliable as a test for mating-type, since the same error of deducing sexual incompatibility from a negative result of a pairing test occurred. A careful study of extensive data published by Christensen (1929, 1931) has revealed that the whole of his findings are in agreement with the hypothesis that heterothallism in *U. maydis* is controlled by a single pair of allelomorphs.

That Hanna and Christensen's 'multiple factors for sex' are in reality factors for weak or deficient pathogenicity is borne out by the fact that as inbreeding was continued, the frequency with which compatible cultures failed to infect the host increased. Thus, when basidiospores derived from widely separated localities were paired, infection resulted whenever spores of opposite mating-type were used (Table 3). When the spores which were paired were derived from the same chlamydospore, the latter being of wild origin, the efficiency of the host-infection test for mating-type dropped to 80 % (Table 4). Finally, when the paired spores were from a closely inbred line in which crossing of spores from the same chlamydospore had been continued for 1-3 successive generations, then only 50 % of the compatible inoculations actually resulted in infection of the host (Table 5). These results are not unexpected if *U. maydis* is normally heterozygous for factors controlling pathogenicity.

Christensen, like Eddins, found that certain single-basidiospore cultures caused infection of the host when injected alone. Since succeeding generations of the fungus following such an infection behaved normally (i.e. single basidiospores did not cause infection), it is clear that the original spores must have been either dikaryotic or diploid. Christensen maintains that these spores were uninucleate, and hence he supposes that the nuclei

Table 3. *Ustilago maydis*. Results of pairing single-basidiospore cultures of opposite mating-type, the individual spores of each pair being derived from wild chlamydo-spores from different localities

References and remarks	Opposite mating-types		Infection	No infection	Total
Hanna (1929, p. 423, Table 6)	C3, 1 and 2	D1, 1 and 2	4	0	4
Minnesota (C) × Italy (D)	D1, 3 and 4	C3, 3 and 4	4	0	4
Christensen (1929, p. 90, Table 1)	Minnesota E	Colorado A, West Virginia A7, Canada A, France A, Hungary A, Italy B, Spain B	7	0	7
	Pennsylvania A, Texas A and B, West Virginia A, France B, Italy A and C, Spain A	Minnesota F	8	0	8
Christensen (1931, p. 149, Table 6, middle)	56, 1 and 3	79, 1 and 3	4	0	4
	79, 2 and 4	56, 2 and 4	4	0	4
Minnesota (56) × Berlin (79) and (85)	56, 1 and 3	85, 1 and 2	4	0	4
	85, 3 and 4	56, 2 and 4	4	0	4
		Totals	39	0	39

Table 4. *Ustilago maydis*. Results of pairing single-basidiospore cultures of opposite mating-type, the individual spores of each pair being derived from the same wild chlamydo-spore

References	Chlamydo-spore	Basidiospores of opposite mating-types		Infection	No infection	Total
Hanna (1929, p. 422, Table 4)	A1	1 and 2	3 and 4	2	2	4
Hanna (1929, p. 432, Table 10)	A2	1 and 3	2 and 4	4	0	4
Hanna (1929, p. 421, Table 2)	B1	1 and 3	2 and 4	4	0	4
Hanna (1929, p. 423, Table 5)	C1	1/2 and 4	3	2	0	2
Hanna (1929, p. 422, Table 3)	C2	1 and 2	3 and 4	4	0	4
Hanna (1929, pp. 421-2, text)	C3	1 and 2	3 and 4	4	0	4
Hanna (1929, p. 421, Table 1)	D1	3 and 4	1 and 2	4	0	4
Christensen (1929, p. 94, Table II)	—	Minn. A13	Minn. B and D	2	0	2
Christensen (1931, p. 153, Table 11 (IV))	52	4	2 and 3	2	0	2
	53	1 and 4	2 and 3	3	1	4
Christensen (1931, p. 149, Table 6 (middle))	56	1 and 3	2 and 4	1	3	4
Christensen (1931, p. 153, Table 11 (I))	77	1 and 3	2 and 4	4	0	4
Christensen (1931, p. 153, Table 11 (III))	78	2/3 and 4	1	2	0	2
Christensen (1931, p. 153, Table 11 (II))	79	2 and 4	1 and 3	2	2	4
Christensen (1931, p. 149, Table 6)	80	1 and 2	3 and 4	0	4	4
Christensen (1931, p. 153, Table 11 (III))	82	1 and 4	2 and 3	4	0	4
Christensen (1931, p. 153, Table 11 (II))	85	3 and 4	1 and 2	4	0	4
		Totals		48	12	60

were diploid. However, it is impossible with one and the same spore, both to stain and count nuclei, and inject it alive into the host plant, and it seems more probable that these spores were dikaryotic, containing both + and - nuclei. Chilton (1943) has also found that certain single-spore cultures of *U. maydis* will cause infection when inoculated singly; they also were presumably binucleate and secondarily homothallic.

Table 5. *Ustilago maydis*. Results of pairing single-basidiospore cultures of opposite mating-type, the individual spores of each pair being derived from the same closely inbred chlamydospore. The chlamydospores were obtained by pairing basidiospores from the same chlamydospore for one, two or three successive generations

Generation	References (Christensen, 1931)	Chlamy- dospore	Basidiospores of opposite mating-types		Infection	No infection	Total
F ₁	p. 149, Table 6	62	2 and 3/4	1	1	1	2
	p. 150, Table 7 (IV)	1	1 and 4	2 and 3	2	2	4
	p. 150, Table 7 (I)	2	1 and 2	3 and 4	4	0	4
	p. 145, Table 3	3	1 and 2	3 and 4	2	2	4
	p. 150, Table 7 (III)	4	1 and 3	2 and 4	2	2	4
F ₂	p. 145, Table 3	5	1 and 3	2 and 4	2	2	4
	p. 145, Table 3	6	1 and 2	3 and 4	2	2	4
	p. 150, Table 7 (IV)	16	1 and 4	2 and 3	2	2	4
	p. 150, Table 7 (II)	17	1 and 3 (4)	2 and 4 (3)	2	2	4
	p. 150, Table 7 (II)	18	3	1/2 and 4	2	0	2
	p. 149, Table 6	39	1 and 3	2 and 4	1	3	4
	p. 150, Table 7 (VI)	41	2 and 4	1 and 3	0	4	4
F ₃	p. 150, Table 7 (III)	10	1 and 3/4	2	1	1	2
			Totals		23	23	46

Sleumer (1932) studied heterothallism in *U. maydis* by two methods. He was able to obtain conjugation of basidiospores on culture medium and used this as a means of determining mating-types, in addition to using the host-inoculation method. He claimed to have found evidence for '6 sex groups'. However, if allowance is made for the fact that a negative result for a mating-type test (made either by the spore-conjugation or the host-inoculation method) does not necessarily imply that the paired cultures are sexually incompatible, then his data agree with the hypothesis that a pair of allelomorphs control the heterothallism. Thus his groups 2, 3, 4 and 6 are evidently of one mating-type and groups 1 and 5 of the other mating-type, as indicated by the pairing reactions shown in his Table 3. Bauch (1932*b*, p. 320) recognized these two mating-types in Sleumer's data and called them B and B¹ respectively.

Bauch (1932*b*) investigated mating-types in *U. maydis* by his modification of the spore-conjugation method, using the presence or absence of aerial hyphae in paired cultures. He found clear evidence for the existence of two mating-types (his B and B¹ strains), but owing to variations in the type of growth after conjugation—'Suchfaden' and 'Wirrfaden'—he claimed that there were six allelomorphs at a second locus (his A, A¹, A², etc.), controlling hyphal character. However, just as with *U. longissima*, there is no justification for regarding the form of the hyphae after conjugation as an essential part of the mating system. Hence Bauch's data support

the hypothesis that heterothallism in *U. maydis* is controlled by one pair of allelomorphs.

Thus the 'multiple factors for sex' in *U. maydis* found by Hanna, Eddins, and Christensen are evidently factors causing a failure to infect the host, while the 'multiple factors for sex' of Sleumer and Bauch are factors causing a failure to produce infection-hyphae ('Suchfaden'). Sleumer found that just those pairs that failed to produce 'Suchfaden' also failed to infect the host. It is evident that the 'multiple sex factors' found by all workers with *U. maydis* are factors for deficient power to infect the host, superimposed on a single pair of allelomorphs controlling mating-type. This is borne out by more recent work. Kernkamp and Martin (1941) found great variation in the pathogenicity of paired haploid lines, and Kernkamp (1942) found that certain pairs of single-spore cultures of opposite mating-type (as shown by pairing with tester strains) failed to infect the host when injected into it. In view of these findings, it is unfortunate that Stakman, Kernkamp, King and Martin (1943) made extensive tests for mating-type by pairing basidiospores from the same chlamydospore. As already demonstrated in discussing Christensen's work, this procedure is likely to result in negative results for the host-inoculation test in certain instances owing to recessive factors for weak pathogenicity, even though the cultures may be sexually compatible. These authors repeated the error of earlier workers by deducing that 'multiple sexual groups' were present.

In all the extensive experimental work on *U. maydis* no evidence has been produced that does not agree with the hypothesis that heterothallism in this species is controlled by a single pair of allelomorphs.

(d) *Sphacelotheca reiliana* (Kühn) Clint. [syn. *Sorosporium reilianum* (Kühn) McAlpine]

Hanna (1929) isolated the four basidiospores produced by a single chlamydospore and showed that the species is heterothallic. Using the host-inoculation technique, he found that infection resulted when spore no. 1 was paired with no. 2 and when no. 3 was paired with no. 4, but all other pairs failed to cause infection. He concluded that four different sexual groups were present. However, just as with *Ustilago maydis*, inbreeding is likely to reveal recessive factors causing feeble growth, and it is not justifiable to conclude from a failure to cause infection that a given pair of spores are necessarily of incompatible mating-types.

Tyler and Shumway (1935) crossed single-basidiospore cultures of *Sphacelotheca reiliana* with *S. sorghi*, and Vaheeduddin (1936) crossed *S. reiliana* and *S. cruenta*. Experimental data from these crosses are not presented in detail, but in the former cross two pairings out of eight and in the latter eleven pairings out of eighteen caused infection of the host. That approximately 50 % of pairings should cause infection implies two mating-types that correspond in the species paired. The existence of simple heterothallism of the plus-minus type is established in *S. sorghi* and *S. cruenta* (Rodenhisser, 1932, 1934, and see (g) below), and there can be no doubt that the heterothallism of *S. reiliana* is similar.

(e) *Sphacelotheca schweinfurthiana* (Thüm.) Sacc.

Bauch (1932a) found that this species is heterothallic, and on the basis of the development of aerial infection hyphae ('Suchfaden') in certain pairings of cultures derived from single basidiospores, he concluded that the heterothallism was tetrapolar. However, on the basis of spore conjugation, bipolar heterothallism was demonstrated, as Bauch admits. It is clear that the chlamydospores studied by Bauch were heterozygous for a pair of allelomorphs affecting the development of the mycelium after conjugation of basidiospores, as well as for the pair of allelomorphs controlling mating-type.

(f) *Sphacelotheca destruens* (Schlecht.) Stev. & A. G. Johns.

[syn. *S. panici-miliacei* (Pers.) Bubák]

Bauch (1934) claimed the existence of two sexual genes and two sterility genes in this species, but did not present the evidence on which this claim was based. It is probable that, as in *S. schweinfurthiana*, the sterility factors control the vegetative mycelium and are not the factors for heterothallism.

(g) *Sphacelotheca sorghi* (Link) Clint.

Rodenhiser (1932, 1934) found clear evidence for the existence of two mating-types in this species and in *S. cruenta*, using the host-inoculation method. Moreover, there was correspondence between + and - strains of the two species. Isenbeck (1935) studied *S. sorghi* using Bauch's modification of the spore-conjugation method and obtained evidence for the existence of only two mating-types, but with inoculation experiments he found slight irregularities in his table of pairing reactions, and concluded that 'multipolar sexuality' existed. However, the irregularities were due to a failure in a small proportion of pairings to obtain infection where it was expected, and this was no doubt due to causes other than sexual incompatibility. Tyler (1938), using both the spore-conjugation method (and Bauch's modification of it) and the host-inoculation method, obtained results similar to Isenbeck, and he concluded there were more than two mating-types present. However, this was due to faulty interpretation of his findings. All his data, like those of Isenbeck, agree with the original work of Rodenhiser and support the hypothesis that heterothallism is determined by a pair of allelomorphs, when allowance is made for negative results to mating-type tests due to causes other than incompatibility.

(h) *Tilletia caries* (DC.) Tul. [syn. *T. tritici* (Bjerk.) Wint.] and *T. foetida* (Wallr.) Liro [syn. *T. levis* Kühn]

Flor (1932) established that *T. caries* and *T. foetida* are heterothallic. He used the host-infection technique and obtained evidence for 'a number of sex groups'. However, negative results were interpreted as implying that the paired cultures were incompatible. Correcting this error, Flor's data are found to be in agreement with a hypothesis that mating-types are controlled by a single pair of allelomorphs. A number of pairs of spores of compatible mating-types failed to cause infection, and this misled Flor into supposing a larger number of mating-types were present.

Moreover, Flor's data show that corresponding mating-types exist in the two species. Thus the basidiospores numbered 209, 213 and 297 of *T. caries* and 223, 285 and 286 of *T. foetida* were evidently of one mating-type (since eight pairings within this group gave no infection) and basidiospores 240, 243, 263, 288, 293, 303, 304 and 306 of *T. caries* and 219 and 308 of *T. foetida* were evidently of the opposite mating-type (since twenty-two pairings within this group gave no infection, and of thirty pairings with the first group twenty-seven resulted in infection). A further thirteen single-basidiospore cultures (nine of *T. caries* and four of *T. foetida*) showed so few positive reactions to the host-infection test when paired with other single-basidiospore cultures (three positive, eighty-eight negative reactions) that their mating-types cannot be determined.

Hanna (1934) obtained similar results: the majority of single-basidiospore cultures were found to belong to one or other of two mating-types, while the remainder showed no infection in any combinations tested. That only two mating-types exist was confirmed by Hanna's studies of spore-conjugation in *T. caries*. The narrow rod-shaped basidiospores (or sterigmata) were isolated, and under suitable cultural conditions were found to conjugate in pairs on the surface of the medium. Whether the basidiospores were derived from the same chlamydospore, or from different chlamydospores, approximately 50% of the pairs showed conjugation. This indicates that the heterothallism is controlled by a single pair of allelomorphs.

Becker (1936) investigated heterothallism in *T. caries* using the host-infection technique. From pairings of the basidiospores from one chlamydospore, he recognized two mating-types, which he called + and -. However, in certain of his pairings of basidiospores from one chlamydospore with those from another, he obtained infection of the host where it was not expected, and he concluded that the situation was more complicated. In his Table 9, the results are shown for fifty-seven different pairings made with sixteen single-basidiospore cultures derived from four widely separated localities (Cosel, Germany; Lyngby, Denmark; Zürich, Switzerland; and Pullman, U.S.A.). If multiple allelomorphs for heterothallism existed, infection would have been expected in all or almost all the pairings. There were five instances where infection was obtained using paired strains supposedly of the same mating-type, but these exceptions are distributed irregularly and do not indicate multiple allelomorphs. Rather do they suggest that two of the cultures (T. 20-3-24 and T. 23-21-6) were dikaryotic, and contained both + and - nuclei. Thus all these investigations support the hypothesis that heterothallism in *T. caries* and *T. foetida* is controlled by a pair of allelomorphs.

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THE SUSCEPTIBILITY OF STRAWBERRY VARIETIES TO RED CORE

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Using a standardized pot infection technique, experiments showed that rapid and reliable estimates may be made of varietal susceptibility to Red Core disease caused by *Phytophthora fragariae* Hickman. Comparison of the pathogenicity of a number of isolates of *P. fragariae* to three varieties of strawberry, Huxley, Perle de Prague and Auchincruive Climax, established the existence of at least three physiologic races within the species.

In a study of the influence of soil moisture and pH on Red Core disease of strawberries, caused by *Phytophthora fragariae* Hickman (Hickman & English, 1951), an infection technique was used for plants in pots that gave rapid and reproducible results. The inoculum consisted of disks which were cut out from the margin of an actively growing colony of the fungus on bean agar and buried just below the soil surface. After inoculation the pots were watered at regular intervals. In the pots which were watered most frequently (twice daily) the progress of the disease was very rapid. Three weeks after inoculation the extent of infection was comparable to that in plants exposed to infection in the field throughout the actively parasitic phase of the fungus from autumn to spring.

Such a test, it was thought, might be used with advantage in the examination of varietal susceptibility, and for rapidly sorting out susceptible from resistant varieties. Accordingly, the same technique was used in experiments made with a number of varieties whose field susceptibility to Red Core was already well known. The isolate of *P. fragariae* was also the same, namely, no. 106, isolated from the variety Madame Lefebvre collected in Cornwall in 1945. The results of one such experiment, after 3 weeks' exposure to infection, are given in Table 1.

Table 1

Variety	Average percentage main root length infected
Perle de Prague	0.0
Early Cambridge	0.5
Oberschlesien	4.7
Duke	14.3
Royal Sovereign	30.6
Cheddar Early	37.2
Huxley	38.3
Madame Lefebvre	58.3

With the exception of the variety Cheddar Early, the field susceptibility of which we have had no opportunity to examine, the results fall well into line with the behaviour of these varieties as observed in the past in the field.

The results of another experiment made to examine the relative susceptibility of several new seedling varieties raised by Mr D. Boyes, Horticultural Research Station, Cambridge, are given in Table 2.

Table 2

Seedling number	Average percentage main root length infected
468	0.0
474	0.0
475	0.0
471	38.8
34	44.2
69	44.5
349	47.8
Huxley	39.7

Such experiments as these demonstrate that susceptibility can be assessed quickly in this way, and the inclusion in each test of one standard variety such as the widely grown susceptible Huxley, enables comparison to be made between the results of separate experiments.

PHYSIOLOGIC RACES

The question of varietal susceptibility and disease control by the use of resistant varieties is greatly complicated in many plant diseases by the existence of physiologic races of the pathogen. Since this work on Red Core was begun evidence has been obtained leading to the recognition of physiologic races in *P. fragariae*.

In the United States of America Scott, Jeffers, Darrow & Ink (1950), in the course of work on the breeding of varieties resistant to Red Core, found that the same varieties appeared to be more susceptible when grown in one State than when grown in another. By growing test varieties in soils collected from different districts they were able to recognize, on the basis of pathogenicity, two races of *P. fragariae*.

In Great Britain, the possibility of the occurrence of physiologic races of the Red Core fungus was first suggested by Reid (1948) as an explanation of the breakdown of resistance in seedling varieties which he had raised. One of these was the variety named Auchincruive Climax which, after 8 years' rigorous testing on contaminated land, was released in 1947 as an immune variety. Its merits soon led to its rapid acceptance in all the strawberry-growing areas of the British Isles. From 1948 onwards outbreaks of Red Core occurred here and there in Climax in Scotland, and through the co-operation of Mr Reid we received material from a number of 'breakdown' outbreaks of Red Core in this and other seedling varieties. Isolations were made from the material, and by means of our precise pure-culture technique we have been able to demonstrate the existence in this country of at least three physiologic races of *P. fragariae*.

The isolates from this 'breakdown' material, together with others obtained in 1945, were tested for their pathogenicity to three varieties of strawberry, namely, the susceptible Huxley, the highly resistant Perle de

Prague, and the originally immune Climax. The plants were watered twice daily and were removed for disease assessment after 3 weeks. The results of four such experiments are given in Table 3.

Table 3

Isolate	Average percentage main root length infected in		
	Huxley	Perle de Prague	Climax
Exp. 1: 106	52.7	1.4	—
R ₄	56.9	38.8	—
R ₆	62.0	49.5	—
R ₁₁	53.8	3.2	—
Exp. 2: 106	51.7	0.7	0.0
R ₄	62.0	51.9	0.0
R ₆	62.4	42.6	0.0
R ₁₁	60.0	1.0	80.9
Exp. 3: 106	34.6	0.2	0.0
105	27.4	31.2	0.0
107	33.2	0.0	0.0
R ₁₂	34.5	0.2	38.5
Exp. 4: 106	63.5	0.0	0.0
106 (reisolate)	66.9	0.0	0.0
R ₁	61.1	28.4	0.0

On the basis of these results the isolates may be grouped into three races as shown in Table 4.

Table 4

Isolate	Source	Pathogenicity to			Race
		Huxley	Perle de Prague	Climax	
106	Cornwall, Mme Lefebvre, 1945	hp	sp	np	1
107	Kent, Huxley, 1945	hp	np	np	
105	Scotland, 1945	hp	hp	np	
R ₄	Scotland, Auchincruive 5, 1948	hp	hp	np	2
R ₆	Scotland, Climax, 1948	hp	hp	np	
R ₁	Scotland, Auchincruive 5, 1947	hp	hp	np	
R ₁₁	Scotland, Climax, 1948	hp	sp	hp	3
R ₁₂	Scotland, Climax, 1948	hp	sp	hp	

hp = highly pathogenic; sp = slightly pathogenic (trace of infection only); np = non-pathogenic.

Race 1, comprising isolates 106 and 107 from English material, is highly pathogenic to Huxley, very slightly pathogenic to Perle de Prague and non-pathogenic to Climax. Race 2 with four isolates from Scotland (105, R₁, R₄ and R₆) is highly pathogenic both to Huxley and to Perle de Prague, and non-pathogenic to Climax. It is curious here that one of the isolates (R₆) was isolated from Climax plants though they were much less heavily infected than the plants from which isolates of Race 3 were obtained. These isolates (R₁₁ and R₁₂), also from Scottish material, were highly pathogenic to Huxley and to Climax but only very slightly pathogenic to Perle de Prague.

DISCUSSION

Testing for varietal susceptibility forms an essential and highly important part of any work on plant-disease control, indicating, as it does, which of the existing varieties may be recommended for use in the presence of the disease, and which are promising sources of resistance for breeding new and better ones. A screening technique that enables resistant and susceptible varieties to be distinguished quickly is obviously an advantage. We believe the method we have described enables this to be done, for it is rapid, simple, and provides a very severe test of resistance to Red Core. Tests of this nature cannot replace field trials but are complementary to them. Field trials are essential for estimating other qualities of a variety, such as its cropping power, season of fruiting, quality of fruit, etc., in addition to disease resistance. But it is not always possible rigorously to test susceptibility to Red Core in the field, for the development of the disease is highly dependent on soil moisture; nor, when comparing a number of varieties, is it possible to guarantee uniform contamination of the land by the pathogen. A further advantage of a susceptibility test such as the one we have described follows on the recognition of physiologic races of *P. fragariae*, for it is a simple matter to use a mixed and standardized inoculum of different races and thus to expose the test variety or varieties to the widest possible range of pathogenicity exhibited by the pathogen. It remains, by further work, to extend our knowledge of physiologic specialization in *P. fragariae*, both as regards the number of physiologic races within the species and their distribution in relation to the distribution of strawberry varieties.

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THE BASIDIOMYCETES OF SKOKHOLM ISLAND

VI. OBSERVATIONS ON CERTAIN UNCOMMON
SPECIES AND VARIETIES

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Descriptions are given of specimens of basidiomycetes found on Skokholm Island, and attributed to the following species:

Leptotus glaucus
Hygrocybe nigrescens
Omphalina abhorrens
Mycena polygramma var. *pumila*
Insiticia amicta var. *iris*
I. clavularis
Tricholoma carneum var. *persicolor*
T. rutilans
Panaeolus fimicola var. *cinctulus*

Panaeolus fimicola var. *ater*
Psilocybe luteonitens
Naucoria embola
N. camerina
Conocybe siliginea var. *ochracea*
Sphaerobolus stellatus
S. stercorarius
S. tubulosus

In the course of ecological observations on the higher fungi of Skokholm Island made during four successive years, a certain number of new, rare, or little known species and varieties have been recorded. For the ecologist a species, other than an apomictic form, consists of a population whose members can and do interbreed freely, together with all other individuals which cannot be separated from this population by morphological criteria. Any demonstrable barrier to crossing (genetical, geographical, or ecological), however incomplete, may be accepted as a species frontier if it is correlated with adequate morphological differences; but in practice the ecologist cannot accept as distinct species two populations, however rigorously they are debarred from interchange of genes, unless their members can be recognized by a not too difficult inspection, preferably out of doors in the rain. In a given locality no two species, in this sense, can possess permanently the same niche; that is, they cannot coexist if precisely the same set of loci are available to each. In such a case one of the two will inevitably prove better fitted to colonize certain of the loci, if not all, and these loci, if sufficiently numerous to support a stable breeding population, will constitute its niche. The peculiar contribution of the ecologist to taxonomy is to supplement the morphological description of a species or other taxon by a specification of its niche, and in particular to discover the differing requirements of related species having the same general habitat.

With the higher fungi, this is a difficult task; the majority of the species are more or less erratic in their fruiting habits, and cannot be detected in the field, much less identified, in the absence of their fructifications. It is therefore only in special circumstances that one can hope for adequate data from which to determine the ecological characters of this or that species; one such circumstance is afforded by the fungus flora of a sufficiently small island.

The species which are described in this paper are mostly not fully established members of the Island community; but their occurrence in such an environment may perhaps help to throw light on their ecological requirements. Every record of the rarer species helps to establish their habitats more exactly, while those forms which are more common but as yet not well defined will in the future be better understood if habitat data are included in their descriptions.

***Leptotus glaucus* (Batsch) Maire**

Very small, of a flaccid almost gelatinous consistency; lateral; odourless. *Pileus* ligulate, convex; minutely silky, sulcate over the gills; glaucous with a whitish bloom when fresh, greyish when dry; flesh thin, flaccid, greyish. *Gills*, primaries one or two only, rather broad, tumid, narrowed toward the base; very irregularly forked but not anastomosing, rather paler than the pileus. *Stem* practically none; pallid becoming discoloured fuscous. *Basidia* cylindrical, *c.* $20 \times 4-5 \mu$. *Paraphyses* (?) up to 30μ long by 3μ wide, apex slightly swollen. *Spores* navicular, hyaline, thin-walled, $6 \times 3 \mu$.

On a dead herbaceous stem (probably *Nepeta* sp.) in the uppermost humus layer under bracken, on a comparatively sheltered cliff slope.

***Hygrocybe nigrescens* (Quél. ex Rea) Karst.**

Medium size, robust, not tall; of a rather fragile consistency. *Pileus* cylindrical then conical, disk soon umbilicate (as in many species of this genus, through failure of the apical hyphae to cohere laterally); smooth, viscid; striate, yellowish, becoming grey and at length black, more rapidly when injured; flesh at first yellow. *Gills* broad, somewhat ventricose, at first appressed to the stem, free; alternate, distant; pale yellow then grey, readily bruising. *Stem* equal; longitudinally striate, viscid; yellowish, extreme base white; flesh scissile. *Cystidia* none. *Spores* elliptical, hyaline, $11 \times 6 \mu$.

Among short grass, within a small ring of *H. miniata*, on ill-drained ground not close to the coast.

Lange (1935-40) seeks to distinguish between *H. nigrescens* and *H. conica* by attributing to the former the 4-spored and to the latter the 2-spored strains; but in the absence of evidence to the contrary this must be assumed to depend on the difference between haploid and diploid fructifications, and has no taxonomic value. Although the two species are closely allied there are definite morphological differences between them; the white base of the stem and the lack of a persistent acute papilla are characteristic of *H. nigrescens*. The form originally described by Quélet (1888) was said to be at first white; perhaps this distinction may be of varietal rank.

***Omphalina abhorrens* (B. & Br.) Quél. (*Omphalia abhorrens* B. & Br.)**

Very small, very stout; rather tough; smell strong like faeces in mature specimens, but faint while young. *Pileus* plane, at length irregularly depressed, margin for a long time somewhat involute; smooth, moist not hygrophanous; fuscous-rufous in colour; flesh fuscous. *Gills* very narrow, decurrent but sometimes appearing scarcely so on account of the contour

of the stem; alternate, not distant; slightly paler than the pileus. *Stem* attenuated downwards, apex strongly expanded; smooth, but wrinkled or channelled; concolorous, base slightly paler; tough. *Cystidia* none. *Spores* allantoid, hyaline, $8-11 \times 4-5 \mu$.

Usually growing two or three together, their bases in contact, but sometimes solitary; mycelium in the second (disintegrated organic) humus layer under the brakes in several parts of the Island.

This species appears to be very rare, but is easily recognized by the smell and the dark colour. *Hygrocybe foetens* has, according to Lange, a different smell, as well as different spores, and the present form has a general appearance quite unlike the *Hygrophoracei*; but it may well be closely allied to *Marasmius impudicus*, though I hesitate to transfer it to that genus while the taxonomy of the latter is still so little agreed.

***Mycena polygramma* (Bull.) Kummer var. *pumila* Lange**

Very small, tall, rather robust; no smell. *Pileus* conical, disk umbonate, margin deflexed; matt, slightly sulcate or wrinkled; blackish, becoming umber, margin paler fuscous, striate; flesh brown, rather tough. *Gills* equally attenuated, adnate; subdistant, alternate, the secondaries very short; greyish brown, edge paler. *Stem* very slightly attenuated upwards; smooth, polished, not striate; somewhat paler than the pileus at least at the apex; flesh brown, flexible, almost tough. *Cystidia* (on edge) subulate, apices acute, hyaline. *Spores* elliptical, with an oil-drop, hyaline; $6 \times 4 \mu$.

Among hypnaceous mosses, and on bare humus (in second layer), under bracken in different parts of the Island, 1949 and 1950.

Although very unlike typical *M. polygramma*, it is true that the differences are mostly in points of minor importance in this genus; its ecology, and in particular whether it can be observed in competition with the larger form, will help to decide whether it deserves specific rank (the combination *M. pumila* is pre-empted). The typical *M. polygramma* has not yet been seen on Skokholm. Mr Pearson informs me that this variety, though but recently recorded in Britain, is not uncommon.

***Insiticia amicta* (Fr.) n.comb. var. *iris* (Berk.) Kühn.**

Very small, very tall and slender; no smell. *Pileus* companulate; smooth but with a fine pruina when very young; greyish striate, paler outwards, extreme margin sky-blue when quite fresh, soon greenish, and white when dry; flesh not continuous with the stem, sharply defined *sub lente*. *Gills* linear, ascending; alternate; grey, paler towards the edge, margin conspicuously white from the cystidia. *Stem* attenuated upwards, base rooting; conspicuously pruinato-striate, apex pulverulent; grey (pruina white), paler downwards, base at first clear blue; brittle, mainly composed of wide hyphae with very few cross-connexions. *Cystidia* smooth, cylindrical, obtuse, apex *c.* 3μ diam. *Spores* elliptical, hyaline, $8-9 \times 5 \mu$.

Mycelium in the second humus layer of the brakes; common.

This form differs from the type almost solely in coloration, but is well worthy of varietal recognition; both forms occur on Skokholm, but the var. *iris* is distinctly later in fruiting. I would strongly uphold the

recognition of *Insiticia** as a genus distinct from *Mycena*; the characteristic anatomy of the stem is a feature fairly easy for the field worker to recognize, and it is likely on theoretical grounds to be one not easily altered in evolution. *Pseudomycena* Cejp is a later synonym.

***Insiticia clavularis* (Fr. ex Kühn.) n.comb.**

Minute, slender, very tall; no smell. *Pileus* hemispherical then convex, smooth, the pellicle containing distinct hyphae; pale grey, striate, the margin almost white; flesh translucent, not continuous with the stem. *Gills* narrow, attenuated outwards, adnate to a low but distinct collar; alternate, rather distant; white. *Stem* cylindrical, base somewhat thickened with a small not distinctly marginate disk; smooth, polished, but with scattered unicellular hairs, especially towards the base; hyaline white. *Cystidia* smooth, inconspicuous, filiform, apex $3-4\mu$ diam., hyaline. *Spores* elliptical, hyaline, $8 \times 4\mu$.

On dead remains of herbaceous plants, especially bracken, in sheltered places.

This species is not uncommon, but was for a long time overlooked; it is easily mistaken for *I. stylobates*. Lange suggests (1935-40) that it may be identical with the *I. stylobates* f. *grisella* Stürm., but this is not the case; this is a mere colour variant, and occurs commonly along with the type on Skokholm, being quite distinct from *I. clavularis*. The present species is probably identical with that described by Rea (1922) under the name '*Mycena dilatata* Fr.', which Kühner (1938), however, attributes to *I. stylobates*. A form resembling *I. clavularis*, but differing in the smaller spores ($4 \times 3\mu$) and fewer gills, has also been found on the Island.

***Tricholoma carneum* (Bull.) Quél. var. *persicolor* (Fr. ex Lange) n.comb.**

Small, robust; no smell. *Pileus* convex, obtuse, margin incurved; very minutely granular, pellicle containing moniliform hyphae; brownish flesh-colour when moist, hygrophanous, pinkish when dry with disk ochraceous, extreme margin pink; flesh white when dry. *Gills* linear, adnate; thin, very crowded, quaternate; edge regular; white. *Stem* equal; smooth, fibrillosely striate in part, apex pruinose; more pink than the pileus, but apex whitish; flesh white, rather elastic. *Spores* narrowly elliptical with rather acute ends, $5 \times 2\frac{1}{2}\mu$.

In grassland, sometimes forming small rings; abundant.

The spores are almost identical with those of *T. carneum*, and smaller than those of *T. ionides*, so that this is evidently not the *T. ionides* var. *persicolor* of Bresadola. The hygrophanous habit makes it very distinct in

* This genus was validly published by Earle (1909), the basynym being *Mycena* sect. *Insititiae* Fr.; but as no one is likely to accept Earle's definition to-day, I emend it as follows:

Insiticia (Fr.) Earle, emendatum. *Mycenae* sensu Friesiano quarum hyphae medullares stipitis latissimae sunt et in carnem pilei non continenter divergent, immo in fasciculo parallelo, apicibus attenuatis vel praemorsis, plus minus recto ad pileum coniunguntur; propter quod pileus a stipite saepissime facilliter secernitur. Speciei plurimae agarici minutissimi sunt. Species typus est *Agaricus corticola* Schum. ab auctore designatus.

Kühner's (1938) use of the name is invalid, as he excludes the type species.

appearance from typical *T. carneum*, but it is not easy to place every specimen in the field, and I cannot think it worthy of more than varietal rank.

Tricholoma rutilans (Schaeff.) Kummer

This common species is only mentioned to record that although it is normally rather strictly confined to coniferous woods, usually being lignicolous, a specimen was found on Skokholm, growing under bracken beside a small stream; no trace of decomposed or buried timber could be found, and the fungus appeared to be growing genuinely in the humus.

Panaeolus fimicola (Fr.) Quél., var. **cinctulus** (Bolt.) Cke.

Small, but rather tall. *Pileus* campanulate, usually somewhat umbonate when expanded; smooth, moist, hygrophalous; reddish brown, with a broad dark brown zone at the margin, all rather paler when dry and then with the extreme margin concolorous; flesh brown. *Gills* ventricose, adnate; quaternate; mottled brown and black, edge whitish. *Stem* equal; smooth; paler than pileus except at the base, apex at first white pruinose. *Cystidia* cylindrical or flask-shaped, apex with thickened wall, hyaline; about 10μ diam. *Spores* elliptical, with an apiculus but with no conspicuous germinal papilla, dark brown by transmitted light; $12 \times 7\mu$.

On horse dung.

Except in point of size (which is not indicated in the original description as the figures are not always natural size in Bolton's drawings) this agrees substantially with *Agaricus cinctulus* Bolt. (1788-91). Specimens were found in 1947, 1948 and 1949, but not in 1950; there can be little doubt that it is an established member of the Island's fungi, though it is probably an obligate dependant on the horse. It seems to be a rare variety, though its colour makes it easy to overlook.

Panaeolus fimicola var. **ater** Lange

Very small, somewhat stout. *Pileus* conico-campanulate, with an umbo; matt, rather coarsely wrinkled; umber brown, blackish when young, dingy brown when dry, of one colour; flesh dark brown. *Gills* as in var. *cinctulus*. *Stem* as in var. *cinctulus*, but darker in colour. *Cystidia* as in var. *cinctulus*. *Spores* rather narrower, $13 \times 6\mu$, same appendages.

On very old horse dung.

This variety has been observed in 1949 and 1950; it does not seem to have been previously described from the British Isles. It is well figured by Lange (1935-40). Although in the shape of the pileus it approaches near to small forms of *P. acuminatus*, it agrees with *P. fimicola* in the absence of a distinct papilla on the spores, in the wide cystidia, and in broad gills; the typical form of the species has not been recorded from Skokholm.

Psilocybe luteonitens (Vahl) n.comb. (*Stropharia luteonitens* (Vahl) Fr.)

Small, slender, tall. *Pileus* convex with an acute umbo; smooth, with a very thick separable viscid pellicle; dull yellow-brown, margin browner; flesh yellow. *Gills* somewhat triangular, broadly adnate with a slight

decurrent tooth; quaternate; blackish brown, edge concolorous. *Stem* equal; smooth, dry, with a very faint ring; ochraceous; slightly rooting among the roots of the grass. *Cystidia* cylindrical, obtuse, c. 4μ broad. *Spores* truly elliptical with no germinal papilla, $13 \times 8\mu$, grey under the microscope.

In a tussock of *Festuca* sp.

This species differs from *P. merdaria* (Fr.) Rick., to which it is closely allied, in the acutely umbonate pileus, with flesh yellow not white, in the stem having a less distinct pith, in the fugacious ring, and in the spores having no distinct germinal papilla; Lange (1935-40) describes a bisporous form with the spores about twice as large by volume. It seems to me that the subgenus *Stercophila*, to which *P. semiglobata* also belongs, is more naturally placed in *Psilocybe* than in *Stropharia*, since the boundary between this group and *Deconica* is very vague, whereas it is separated from *Stropharia* proper both by the stem structure, with a more or less differentiated pith, and by the development of the gills, whose free edge increases from less than half the arc of attachment to nearly equal with it, whereas in *Stropharia aeruginosa* the ratios are from $\frac{3}{4}$ (before rupture of veil) to about $1\frac{1}{4}$ (maturity).

***Naucoria embola* (Fr.) Quél.**

Very small, stout, tall. *Pileus* hemispherical, then convex; very smooth, dry; dull yellow, somewhat striate when moist but scarcely changing colour; flesh pale yellow. *Gills* broad, obtusely decurrent with a straight edge; distant; concolorous, becoming more rufous. *Stem* expanded upwards; smooth; slightly paler than the pileus. *Cystidia* cylindrical or somewhat bottle-shaped, apex obtuse. *Spores* somewhat bean-shaped, $10 \times 6\mu$, yellowish by transmitted light, cinnamon in powder.

Among *Potentilla* spp. in boggy ground, humicolous.

This is a very distinctive species; Mr Pearson informs me that it is probably fairly common, at least on the west coast.

***Naucoria camerina* (Fr.) Quél.**

Minute, tender. *Pileus* convex, papillate; smooth, moist, pellicle made up of coarse brownish hyphae; bright brown, margin striate, papilla with a somewhat hyaline appearance; flesh brownish. *Gills* very ventricose, adnate; alternate, subdistant, concolorous. *Stem* equal; lower part with a partial coating of white fibrils; concolorous, darker towards the base. *Cystidia* subulate, with a ventricose base (immersed), apex usually subcapitate; protruding about 20μ . *Spores* elliptical, $8 \times 6\mu$, brownish yellow by transmitted light.

On fragments of bracken in the upper layer of humus in the brakes.

These specimens have a superficial resemblance to *G. nana*, which occurs in like habitats on the opposite coast, though it has not been seen on Skokholm; the present species lacks the characteristic cystidia of *G. nana*. Although much smaller than the typical form of *G. camerina*, this may perhaps be attributed to the untypical habitat; the microscopic characters show no difference.

Conocybe siliginea (Fr.) Kühn. var. **ochracea** Kühn.

Small, slender, tall. *Pileus* semi-ovate, not much expanding; smooth, with hymeniform pellicle; brown, scarcely striate, ochraceous when dry; flesh light brown. *Gills* vesicate (bi-segmental) rather broad, ascending; alternate, not much crowded; ferruginous, darker than the dry pileus. *Stem* equal, slightly thickened at the base; very smooth, minutely striate with lines of hairs; pallid, striae white; solid. *Cystidia* (on gills) capitate, body 10μ broad, neck very short, head nearly spherical 3μ diam. *Spores* elliptical with slightly sharp ends, not quite symmetrical; $8 \times 6\mu$ (1947) or $12 \times 8\mu$ (1950). (Kühner describes specimens with both sizes of spore.) *Hairs* on stem cylindrical, somewhat nodulose, often matted.

Among grasses in drier situations.

This variety does not seem to have been previously recorded in the British Isles, at least not under this name; it has been well characterized by Kühner (1937), and if attention is paid to the colour of the stem, and the absence therefrom of capitate cystidia, it cannot easily be mistaken for any other *Conocybe*, though it is superficially much like *C. tenera* and it may in the past have been passed over as such.

Sphaerobolus stellatus (Tode ex Lorenz) Pers.

Minute, globose, *c.* 2 mm. diam., densely gregarious. *Peridium* opening by 5-7 acute regular teeth; rough or hairy; externally pale wood colour or dingy yellowish. *Endoperidium* pellucid, appearing at first pale yellowish but soon exposing a bright orange zone. *Gleba* yellowish when first exposed, but orange-brown before expulsion. *Spores* elliptical, $8-9 \times 5\mu$.

Growing on a very wide variety of substrates.

Sphaerobolus stercorarius Fr.

Minute, rather irregularly globose, *c.* $1\frac{1}{2}$ mm. diam., in twos and threes. *Peridium* dehiscing by 3-5 unequal and usually obtuse teeth; rugged and incrustated with fragments of the substrate; whitish. *Endoperidium* with a distinct orange zone, but paler than in *S. stellatus*. *Gleba* pallid, becoming translucent greenish or yellowish ochraceous. *Spores* globose, $6-8\mu$.

On dung of rabbits.

Sphaerobolus tubulosus Fr. ex Lorenz

Minute, oval or urceolate, *c.* 2×3 mm., densely gregarious. *Peridium* dehiscing by 5-7 unequal teeth; hairy becoming smooth; rufescent whitish, or pure white. *Endoperidium* pallid, with a not very conspicuous orange zone. *Gleba* pale brown. *Spores* allantoid, $9-11 \times 5-7\mu$.

On dung of sheep (originally described from pine twigs, but as Fries (1832) says it is 'solitary' this is presumably a marginal habitat).

I make no apology for describing these three species, for as far as I know there is no adequate description of them in English. The genus *Sphaerobolus* has been investigated by Lorenz (1933) with the help of cultural methods, and though he did not secure material of all the species alleged to exist,

he showed clearly that many of these were merely forms of *S. stellatus*. He found *S. tubulosus* to be distinct, and he distinguished the genuine diagnostic characters from the spurious. The description given by Rea (1922) of *S. stellatus* is a mixture of these two species, and his *S. terrestris* is much the same. Lorenz rejected *S. stercorarius*, but he did not know the spore characters which Fries, as usual, omitted from the description; there is no doubt that by the criteria established by Lorenz this is a good species. All three species occur on Skokholm, and the two last are (at least nominally) new to the British Isles; *S. stellatus* is, as everywhere, the commonest.

I wish to thank Mr A. A. Pearson for many helpful criticisms and suggestions.

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THE SPECIES OF *USNEA* IN GREAT BRITAIN AND IRELAND

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Based on the classification of Motyka, a key is given to forty-eight species, one subspecies and four varieties of the genus *Usnea*, found in Europe, with special reference to those of the British Isles.

The lichens often found on trees and commonly known as 'beard moss' or 'old man's beard', belong to the genus *Usnea*, and their specific names have been used in various ways both by British and other authors. The Polish lichenologist, Dr J. Motyka, many years ago, devoted himself to an examination of the genus and examined thousands of specimens in his own country and in the various herbaria of Europe, including those in our own British Museum (Natural History) at South Kensington.

In 1936-8 he published his *Lichenum generis Usnea studium monographicum* in three volumes of 651 pages with descriptions in Latin of 449 species, including forty-three species which had been previously placed under *Neuropogon*, *Letharia*, *Chlorea* or *Eumitra*. Of these species 285 are not European, so that only 164 are European, and many of these have not been found in the British Isles.

The method adopted by Motyka was of such a 'splitting' character that his work was not accorded so much credit as it was worth. It certainly created species on slight differences and was destructive of much previous work, but, on the other hand, drew attention to some mistakes which had been made in the interpretation of some of our common species, especially in regard to *U. hirta*, *U. dasypoga*, *U. florida* and *U. ceratina*. In our own country Miss A. L. Smith (1918) in her *Monograph of the British Lichens* followed Heber Howe (1912) in regard to nomenclature, using *U. barbata* and *U. plicata* where the names of *U. dasypoga* and *U. ceratina*, as used by Crombie (1894), were respectively preferable. (*U. barbata* (L.) Wigg. emend. Mot. and *U. plicata* (L.) Wigg. have been retained by Motyka for some *Usneas* with very limited distribution in Scandinavia.) In later years she acknowledged her mistake in doing so. Some *Usneas* described by Stirton (1881-2) during the last century and neglected in our British lichen manuals have been rescued from their oblivion and included by Motyka in his monograph.

During the last fifteen years since Motyka's work was published, I have attempted to use his methods in extensive field-work and to name the plants accordingly. Instead of five species and two varieties (which have been since elevated by Motyka to specific rank) as in the Monograph (A. L. Smith, 1918), there are now more than thirty species recorded for our islands.

Lamb (1939) has criticized Motyka's work on the *Neuropogon* group, and many other criticisms have been made by other authors of his work on the

true *Usneas*. Motyka relies largely on the usual methods used in the determination of *Usnea* species: size, erectness or pendulosity, colour when fresh and after keeping in the herbarium, rigidity or softness, presence or absence of soralia or isidia, reaction to potassium hydrate solution, amount of branching and presence or absence of articulations, papillae, tubercules, verrucae, foveolae and pseudo-cyphellae. In addition to these characters he attaches importance to the coloration at the base, the relative thickness of the main branches and the apices, the relative sizes of the cortex, medulla and axis, the laxity or compactness of the medulla and its reactions with potassium hydrate or iodine and to the corticolous or saxicolous habitat.

My field work has shown me that there is a fair amount of differentiation in some of these characters for very definite reliance to be placed on them. It has also shown me that the most frequent *Usnea* in our islands is *U. comosa* (Ach.) Röhl., which had been placed by most British authors as *U. hirta* or *U. florida* var. *hirta*, whereas Motyka considers that *U. hirta* (L.) Wigg., as emended by him, is a plant with a paler base, usually more or less foveolate and longly isidiose in the upper parts. In a recent expedition to the woods about Bodmin Moor, Cornwall, scarcely any *Usnea* other than *U. comosa* could be found. In Somerset it is certainly the most abundant species, and it has been recorded from more botanical vice-counties than any other species of *Usnea*.

It is a difficult problem to make a key to the British species of *Usnea*, but I have found the following very useful. Some latitude must be allowed in most keys but some additional latitude must be allowed in a key to *Usnea*.

All the species given in this key are European and may be British. Those which are not known to be British have their names in brackets []. The number in parentheses () following the specific name is the page reference to Motyka's (1936-8) *Monographicum*. When the wording of the key involved one or more alternatives the qualifying word (usually *or*) or words is (or are) given in italics.

The value of using the coloration with a solution of potassium hydrate (K) as a distinguishing character is not great and must not be relied on unduly.

The value of using as a distinguishing character the relative sizes of the width of the cortex to the width of the medulla and to the diameter of the axis (expressed by C: M:A, where C is taken as 1) must not be estimated too highly. These relations are somewhat variable according to the position and sizes of the branch tested, and it is advisable to test branches from a similar position on the plant. For comparative purposes it is better to test thick branches near the basal part of the plant. The relative thickness of the medulla to the diameter of the axis is often more useful than when the cortex is taken as 1, as the distinction between cortex and medulla is not always clear and easy to estimate. The ratios are not always so definite as given in the key and are only approximate, even in specimens authenticated by Motyka. When distinguishing plants with farinose (finely powdered) soredia from those which are isidiate (cylindrical) young soralia should be observed. In the key Motyka's work has been largely followed, though I am not an advocate of the excessive differentiating methods which he has adopted, especially in the *comosa* and *sorediifera* groups. There is a tendency

in the *comosa* group for the isidiate soredia to become erose and appear to be farinose. In the *sorediifera* group there is too much segregation; Motyka himself finds *U. wasmuthii* and *U. monstrosa* difficult to accept and is doubtful about *U. laricina* as some other species have branches which are flexuose.

KEY TO *USNEA*

- 1 *a.* Axis, at least in the thicker branches, hollow. The only British species with a hollow axis has the thallus terete, greyish green, with an axis occupying nine-tenths of the diameter and with a large cavity. ***U. implicita*** (Stirt.) Zahl. (*M.* 60)
- 1 *b.* Axis centrally solid or so little lacerate that the cavity is never distinct. 2
- 2 *a.* Thallus not exactly terete but foveolate or scrobiculate, elongate (except in short forms of *U. hirta*), soft, epapillose, not darkened at base, medulla lax. 3
- 2 *b.* Thallus either $\pm$ exactly terete or acutely angular or rigid. 6
- 3 *a.* Thallus elongate (to 30 cm.), much longer than broad, never fruticulose, always very soft, pallid or greyish green. C : M : A about 1 : 5 : 6. ***U. cavernosa*** Tuck. (*M.* 77)
- 3 *b.* Thallus moderate or small, not or little longer than broad, at base attenuate, sorediate, medulla lax or very lax, K—. 4
- 4 *a.* Thallus up to 15 cm. with thickish branches (to 1 mm.), all flaccidly pendulous and much foveolate, very soft; soredia shortly isidiose and not conspicuous; medulla very lax. C : M : A about 1 : 2.5 : 2.5. ***U. foveata*** Vain. (*M.* 82)
- 4 *b.* Thallus smaller, usually under 8 cm., not distinctly foveolate in the whole length of the thallus. 5
- 5 *a.* Thallus small, 1-8 cm., not papillose, soredia usually many and longly isidiose and in the upper parts giving the 'hirta' or spinulose appearance. C : M : A 1 : 3-5 : 4-5. ***U. hirta*** (L.) Wigg. emend. Mot. (*M.* 83)
- 5 *b.* Thallus 4-7 cm. with largish semi-globose soralia; when fresh greyish green, becoming reddish brown in the herbarium. ***U. subcornuta*** Stirt. (*M.* 81)
- 6 *a.* Thallus elongated to 30 cm. or even more, pendulous, soft, neither rugose nor tuberculated; articulated into short segments; branchlets few or none; pseudocypbellae always present but sometimes indistinct; soredia absent or almost so; medulla lax, K—, with radiating hyphae. C : M : A 1 : 5-18 : 5. ***U. articulata*** Hoffm. (*M.* 115)
- 6 *b.* Thallus elongated or short, continuous or if articulated without pseudocypbellae. 7
- 7 *a.* Thallus in herbarium soon becomes fuscescent or persistently yellowish or straw-coloured but is never deep greyish green. When fresh the plants may be distinguished by the thin papyraceous cortex or if this is thickish (as in *U. subpectinata* and *U. inflata*) by being typically smooth or smoothish. 8
- 7 *b.* Thallus in herbarium not changed or only becoming fuscous (obscurely) after a long time and then the plants are $\pm$ papillose. 12
- 8 *a.* Thallus with $\pm$ inflated segments. Medulla lax or very lax, as wide as or usually wider than the diameter of the axis 9
- 8 *b.* Thallus inflated or not. Medulla fairly lax, much narrower than the diameter of the axis. 11
- 9 *a.* Soralia rather large, farinose, becoming broadly efflorescent. Thallus suberect or subpendulous, 3-4 (-7) cm. Medulla very lax, K—, though it may become somewhat reddish after some time. On trees. Cortex about 35 μ . C : M : A about 1 : 7 : 5.5. ***U. glabrata*** (Ach.) Wain. (*M.* 495)
- 9 *b.* Soralia much smaller and less distinct. Medulla lax or very lax, with K+ yellowish, or $\pm$ reddish. On trees or rocks. 10

- 10a. On trees. Branches somewhat inflated with obtuse and often incurved apices, usually distinctly papillose, sordid-glaucous-green when fresh, in herbarium lurid-fuscos. Cortex about 100 μ ; C:M:A about 1:4:2. Medulla lax, K+ yellowish, later subrufescent. **U. subpectinata** Stirt. (*M.* 508)
- 10b. On trees. Branches distinctly inflated with sharper and straighter apices, indistinctly papillose, paler when fresh, in herbarium often brighter brownish. Cortex about 30 μ . C:M:A about 1:10:5.5. Medulla very lax, K+ intense red, I+ subcoerulescent. **U. intexta** Stirt. (*M.* 505)
(*U. horrescens* (Tayl.) Mot., as given by Porter (1948), refers to *U. intexta* Stirt.)
- 10c. On rocks. Plant of more compact habit than *U. intexta* and more frequently branched. Cortex about 40 μ . C:M:A about 1:7:5.5. Medulla lax, K+ reddish, I+ subcoerulescent. **U. cornuta** Krb. (*M.* 509)
- 11a. On trees. Thallus 4–7 cm. long with inflated segments; in herbarium reddish fuscos. Cortex about 80 μ . Medulla fairly lax, C:M:A about 1:2.5:5. **U. inflata** Del. (*M.* 510)
- 11b. On trees (or rock). Thallus 4 cm. high with segments not inflated, in herbarium brownish. Cortex about 50 μ . Medulla somewhat lax. C:M:A about 1:2.4:4.5. **U. flammea** Stirt. (*M.* 563)
- 12a. Thallus distinctly 'barbatus', often intensely green, almost always slenderly papillate. Medulla usually lax or rather lax ($\pm$ compact in *comosa*, *distincta*, *florida*, *glabrescens* and *wasmuthii*); if relatively thin (as in *comosa*, *diploypus*, *distincta*, *florida*, *fulvoreagens*, *glabrescens*, *laricina* and *wasmuthii*) the plant is boreal. **13**
(‘Barbatus’ may be taken as meaning bearded.)
- 12b. Thallus not distinctly ‘barbatus’, sometimes even little branched and little papillose, not usually intense green. Medulla compact (sublax in *marocana* and absent or almost so in *longissima*). Common in the temperate zone. Chiefly *ceratina* and *rubicunda* groups. **34**
- 13a. Thallus elongated and pendulous, much longer than broad, almost always isidiate; if sorediate the soredia are not on apical portions. **14**
- 13b. Thallus short, mostly erect, rarely over 10 cm. long. When moderately long, as in *extensa*, with farinose soredia; if fertile the apothecia are $\pm$ terminal. Apices usually provided either with apothecia or soredia. **19**
- 14a. Thallus usually fertile and esorediate, 20–30 cm., darkish-glaucous-green and thinly provided with very obtuse tubercles. Branches 1–1.5 mm. thick and little attenuated at their apices. Medulla (and also the axis) is 8–10 times as thick as the cortex. Widely distributed on trees in Europe. **U. glauca** Mot. (*M.* 167)
- 14b. Thallus 15 cm. long with a firm and strict appearance, usually purer green, smooth or finely papillate. Branches 1.2 mm. thick, gradually attenuated at the apices. Medulla (and also the axis) is about three times as thick as the cortex. Almost always fertile with cupuliform and rather small apothecia seated on the lateral branches. **U. rigida** (Ach.) Röhl. (*M.* 177)
- 14c. Thallus sorediate (rarely also fertile with apothecia usually terminal), often over 20–30 cm. long, soft with few, often thin, branches, usually papillose or tuberculate. *U. dasyypoga* group **15**
- 15a. Thallus capillaceous, less than 0.7 mm. thick, little or not papillose. **16**
- 15b. Thallus usually thicker, more than 1 mm. thick and tapering to the apices; primary branches usually more or less tuberculate. **17**
- 16a. Thallus subarticulate, to 30 cm., typically epapillose though sometimes partly papillose but not tuberculate (or very little). **U. capillaris** Mot. (*M.* 185)
- 16b. Thallus subcontinuous, to 25 cm., with somewhat scattered papillae; tuberculate with isidiate soralia exrescent from the tubercules. **U. fibrillosa** Mot. (*M.* 188)

- 17a. Thallus up to 30 cm. long. Primary branches minutely papillose; secondary branches elongated, little papillose and relatively grossly tuberculate with obtuse tubercles, mostly only tuberculate. Soredia isidiate. C : M : A 1 : 2.5 : 4.2.
U. dasypoga (Ach.) Röhl. (*M.* 189)
- 17b. Thallus up to 25 cm. or more. Branches for the most part acutely papillose, but tubercles are indistinct or absent or very rare. Soredia isidiate or absent. **18**
- 18a. Thallus up to 25 cm., rarely articulate, pale greyish green, always with branchlets many and longish, indistinctly tuberculate, Medulla K—, about the same width as the diameter of the axis. C : M : A about 1 : 4.7 : 5. Soredia isidiate.
U. muricata Mot. (*M.* 219)
- 18b. Thallus up to 30 cm. or more, articulate, pale green when fresh but in herbarium somewhat straw-coloured, acutely papillose. Primary branches ± uniform from base to apex. Soredia absent. Medulla K+ reddish. C : M : A 1 : 5.6 : 7.
U. chaetophora Stirt. (*M.* 186)
- 19a. (from 13b). Thallus typically fertile, esorediate. *U. florida* group. **20**
- 19b. Thallus sorediate, very rarely also fertile. **22**
- 20a. Thallus short and broad, 5–8 cm., little longer than broad, greyish green both when fresh and in herbarium, branches less than 2 mm. thick. Medulla K— or faint. Apothecia terminal on all or most branches. **U. florida** (L.) Wigg. (*M.* 236)
- 20b. Thallus rather elongate, 10–30 cm. and pendulous. Primary branches partly sterile. Medulla K— usually lax. **21**
- 21a. Medulla subcompact, usually less than half the thickness of diameter of axis. Thallus olivaceous-greyish-green, up to 25 cm. long. Apothecia about 1 mm. in diameter, situated on the apices of lateral branches. Cortex about 60 μ. C : M : A 1 : 2 : 5.3.
[**U. hapalotera** (Harm.) Mot.] (*M.* 235)
- 21b. Medulla lax, about as thick as diameter of axis. Thallus obscurely green, 20–30 cm., with longish papillae. Apothecial disk somewhat caesious. Cortex about 100 μ. C : M : A 1 : 3.5 : 3.5.
[**U. montana** Mot.] (*M.* 234)
- 22a. Soredia always isidiose (cylindrical) except in abnormal cases (e.g. the soredia may, especially in herbarium specimens, be erose and appear farinose).
U. comosa group. **23**
- 22b. Soredia farinose (powdery); rarely some isidia present. Young soralia must be observed.
U. sorediifera group. **26**
- 23a. Thallus irregularly branched, at base erect, in upper portion often prostrate, rarely fractured. Soredia longish, in soralia, or often placed singly. Plant epilithic, up to 5 cm. long. Cortex about 100 μ; medulla about 170 μ, K+ slightly yellowish and then somewhat reddish; axis about 500 μ.
U. diplotypus Vain. (*M.* 257)
- 23b. Thallus always regularly fruticose; if epilithic distinctly articulates constricted. **24**
- 24a. Thallus not compressed, epilithic, 5–8 cm. long, distinctly articulates constricted. Cortex about 60 μ, medulla about 280 μ, lax, K—, axis about 180 μ.
U. fragilesceus Hav. (*M.* 259)
- 24b. Thallus compressed, divergently branched, on trees or wood, though some forms of *U. comosa* may sometimes occur on rock. Plant usually about 4–7 cm. long. **25**
- 25a. Thallus usually pure green (or pale-olivaceous), with thickish (to 1.5 mm.) primary branches which are rather soft and minutely papillose. Secondary branches attenuated at base and distinctly inflated in the portions (segments formed by articulations) above. Cortex about 40 μ, softish; medulla about 220 μ, lax K— and then slightly reddish; axis about 200 μ.
U. mollis Stirt. (*M.* 259)

- 25*b*. Thallus usually greyer in colour with primary branches harder and more papillose. Secondary branches not attenuated at base and not inflated above, rarely fractured. Cortex about 100 μ , rigid, medulla about 200 μ and more compact, K— or yellowish to reddish, Axis about 380 μ . Pale carneous cephalodia are frequently present.
U. comosa (Ach.) Röhl. (*M.* 264)
(*U. hirsutula* Stirt., recorded from Worth Forest is placed by Motyka as *U. comosa*. *U. subfloridana* Stirt. is not accepted by Motyka. He thinks that it may be placed under the small sordid form of *U. comosa* which he calls subsp. *sordidula*.)
- 26*a*. (from 22*b*). Thallus always rather short, 3–10 cm., erect or subpendulous, divergently fruticulose. 27
- 26*b*. Thallus elongate, to 15 cm., pendulous, at least twice as long as broad. 33
- 27*a*. Thallus always flexuose, about 6 cm. long and almost as broad as long, soft green with basal branches to 1 mm. thick. Both branches and branchlets are distinctly flexuose. Soralia rather minute, convex, distinctly granulose and largish papillae in the upper parts may become sorediate. Cortex about 60 μ . C:M:A 1:2:5.
U. laricina Vain. (*M.* 280)
- 27*b*. Thallus less flexuose or with thickish (about 1.5 mm.) straight or nearly straight branches. 28
- 28*a*. Apical portions of thallus short and little distinct or soralia broadly eroded. 29
- 28*b*. Apical portions elongate and gradually becoming attenuated though *U. distincta* may be rather abruptly acuminate. Soralia minute and definitely limited. 32
- 29*a*. Thallus when fresh intensely green and also so in herbarium or, if pale, longly papillose. Soredia efflorescent in globose or subglobose heads. Plant short, compact (fruticose caespitose) about 4–5 cm. Cortex about 50 μ , rather soft, medulla about 200 μ , rather lax; K—, axis about 300 μ .
U. compacta Mot. (*M.* 294)
- 29*b*. Soredia in more or less eroded soralia. 30
- 30*a*. Medulla appreciably yellowish and then reddish with K (sometimes rather vague in *U. monstrosa*). 31
- 30*b*. Medulla K—. Thallus $\pm$ obscurely green, 5–7 cm. high, rather broad with branches up to 1.8 mm. thick and branchlets divergent, almost appearing inflated and with many minute papillae. Cortex about 60 μ . C:M:A 1:3.7:3. (Note that axis is less than medulla.)
U. sorediifera (Auct.) Mot. (*M.* 286)
- 31*a*. Thallus $\pm$ regularly fruticulose, 6–10 cm. long, and broad with branches 1.2 mm. thick. Cortex about 80 μ , hard and rigid; M 150–180 μ , lax; A 400 μ .
U. fulvorenans (Räs.) Mot. (*M.* 284)
- 31*b*. Thallus very irregularly branched, about 6 cm. long, with branches about 1 mm. thick and irregularly flexuately curved. Cortex about 60 μ ; M 150 μ , lax; A 300 μ .
U. monstrosa Vain. (*M.* 279)
(Motyka is not convinced of the autonomy of *U. monstrosa*, though he is of its monstrosity. He thinks that it may be an abnormal variation of some other species. Motyka considers that *U. wasmuthii* is difficult to accept as a distinct species. The rejection of these two species seems to be justified by my experience. In some material collected by R. Burn on Cadar and examined by me most of the material belonged to *U. fulvorenans*, but some *U. monstrosa* and *U. wasmuthii* appeared to be present.)
- 31*c*. Thallus 8–10 cm. long with apices relatively long and becoming capillaceous. Cortex about 40 μ and rather soft, axis about 60 μ and compact, axis about 180 μ .
U. wasmuthii Räs. (*M.* 281)
- 32*a*. Medulla thin, about 150 μ , K—, its thinness being relative to the axis, the diameter of which is more than twice the width of the medulla. Plant rigid (firm and elastic), subpendulous, up to 10 cm. long, greyish green to straw coloured. Apices little branched.
U. distincta Mot. (*M.* 298)

- 32*b*. Medulla relatively thin (about 180 μ whilst the diameter of the axis is about 380 μ), lax, K+ reddish (K- in variety *glabrella*). Plant greyish green to 10 cm. long and broad. Apical portions elongate and usually simple (unbranched).
U. glabrescens (Nyl.) Vain. (*M.* 299)
- 33*a* (from 26*b*). Thallus compressed, with the soredia dispersed on the upper parts right to the apices. Medulla about 150 μ usually K+ yellowish and then reddish (K- in var. *gemina*). Thallus, when fresh, greyish green and becoming pale-ochroleucous in the herbarium.
U. extensa Vain. (*M.* 303)
- 33*b*. Thallus with the soralia situated on the primary branches. Medulla compact, K+ red. C : M : A 80 : 80 : 330 μ .
[**U. silesiaca** Mot.] (*M.* 289)
- 34*a* (from 12*b*). Medulla very thin, not rosy. Plant rather short, usually densely provided with branches. Cortex may be reddish for some species or forms. On trees or rocks. 35
- 34*b*. Medulla usually rosy, or plant very elongated, often over 30 cm. On trees. 37
- 35*a*. Thallus almost smooth (or verrucae very inconspicuous), erect, 4-6 cm. long; isidia slender. Branch about 0.5-0.6 mm. thick. C : M : A about 1 : 2 : 2. Medulla with iodine indistinctly bluish. On rocks in littoral regions.
U. rupestris Mot. (*M.* 323)
- 35*b*. Thallus distinctly red or reddish or with reddish colour visible in section. Usually on trees. 36
- 36*a*. Thallus suberect, usually about 7 cm. long, distinctly red or reddish or spotted with red or rosy colour but not lurid brownish. Soredia small, isidiate. Medulla yellowish with K usually (not in var. *insensibilis* Mot.) and may be indistinctly bluish with iodine, about 150 μ , little thicker than the cortex and less than half as thick as the axis.
U. rubicunda Stirt. (*M.* 339)
(*U. rubicunda* has been given as *U. rubiginea* (Michx.) Mass. in some of my previous work; *rubicunda* as a specific name has priority.)
- 36*b*. Thallus pendulous, about 15 cm. long, lurid brownish or sordid reddish, but the rosy colour is usually visible only in section. Soredia finely isidiate. Medulla compact as in *rubicunda* but reaction with K may be redder. C : M : A about 1 : 1.7 : 4.6.
U. sublurida Stirt. (*M.* 349)
(*U. protensa* Stirt. is included by Motyka under *U. sublurida*.)
- 37*a*. Medulla intensely rosy, sublax, rather thicker than the diameter of axis, K-. Thallus short, 6 cm. high and broad, pale-greyish-green, irregularly verruculose and with isidiate soredia.
U. marocana Mot. (*M.* 359)
- 37*b*. Thallus typically pendulous and elongated. 38
- 38*a*. Thallus about 15-40 (-80) cm. (less in subsp. *incurviscens* (Arn.) Mot.), pale-greyish-green, irregularly provided with verruculose papillae which are largish and farinously decorticated at apices. Soredia isidiate, sometimes almost like branches. The farinously decorticated papillae may be mistaken for isidia which are less frequent than these. Medulla white with a rosy tint, K- or + yellowish, not so thick as diameter of axis.
U. ceratina Ach. (*M.* 365)
- 38*b*. Thallous pendulous, up to 6 m. long with almost uniform branches, destitute (or almost) of cortex, medulla very thin, the axis thus occupying almost the whole width. I+ blue. Not known from the British Isles.
[**U. longissima** Ach.] (*M.* 423)

INDEX TO THE KEY AND DISTRIBUTION IN THE BRITISH ISLES

The first number gives the number of the plant in the key, whilst the second number gives the number of botanical vice-counties (England and Wales, 71; Scotland, 51; Ireland, 40; Channel Islands, Total 153) in the British Isles in which it has been found.

Name	Key	No. of vice- counties	Name	Key	No. of vice- counties
<i>articulata</i>	6a	16	<i>horrescens</i>	10b	—
<i>barbata</i> (see pp. 368-9)		—	<i>incurviscens</i> (var.)	38a	—
<i>capillaris</i>	16a	?	<i>inflata</i>	11a	8
<i>cavernosa</i>	3a	4	<i>implicita</i>	1a	1
<i>ceratina</i>	38a	23	<i>insensibilis</i> (var.)	36a	—
<i>chaetophora</i>	18b	1	<i>intexta</i>	10b	19
<i>comosa</i>	25b	66	<i>laricina</i>	27a	3
<i>compacta</i>	29a	8	<i>longissima</i>	38b	0
<i>cornuta</i>	10c	3	<i>marocana</i>	37a	?
<i>dasyopoga</i>	17a	17	<i>mollis</i>	25a	20
<i>diplotypus</i>	23a	1	<i>monstruosa</i>	31b	1
<i>distincta</i>	32a	10	<i>montana</i>	21b	0
<i>extensa</i>	33a	9	<i>muricata</i>	18a	9
<i>foveata</i>	4a	1	<i>plicata</i> (see p. 368)	—	—
<i>fibrillosa</i>	16b	1	<i>protensa</i>	36b	—
<i>flammea</i>	11b	13	<i>rigida</i>	14b	1
<i>florida</i>	19-20	23	<i>rubicunda</i>	36a	20
<i>fragilescens</i>	24a	3	<i>rubiginea</i>	36a	—
<i>fulvoreaegens</i>	31a	22	<i>rupestris</i>	35a	9
<i>gemina</i> (var.)	35a	—	<i>silesiaca</i>	33b	0
<i>glabrata</i>	9a	5	<i>sorediifera</i>	30b	4
<i>glabrella</i> (var.)	22b	—	<i>sordidula</i> (subsp.)	25b	—
<i>glabrescens</i>	32b	11	<i>subcornuta</i>	5b	1
<i>glauca</i>	14a	1	<i>subfloridana</i>	25b	—
<i>hapalotera</i>	21a	0	<i>sublurida</i>	36b	10
<i>hirsutula</i>	25b	—	<i>suspectinata</i>	10a	6
<i>hirta</i>	5a	11	<i>wasimuthii</i>	31c	1

The eastern counties have relatively few *Usneas*, in fact, Sowter (1950) states that only four species have been recorded from Leicestershire where they are now almost extinct. In the south-western counties they are still frequent, twelve species have been recorded for Somerset and twenty-one for Devon.

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THE ECOLOGY OF SOIL FUNGI

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(With 8 Text-figures)

The influence of soil type and soil horizon on the distribution of microfungi in five natural grassland soils at Lakenheath Warren has been investigated. The soils, which are all very dry and sandy, vary from a shallow, highly alkaline soil to a deep, highly acid podsol.

One hundred and forty-eight species of fungi have been isolated from these soils by the soil-plate method. The most common genera are *Penicillium* and *Mortierella*, followed by *Absidia*, *Cephalosporium*, *Fusarium*, *Gliocladium*, *Gliomastix*, *Mucor*, *Thielavia*, *Trichoderma* and *Zygorrhynchus*. The occurrence of fungalspecies in twenty profile samples of each soil has been recorded, and the distribution of the more important species is discussed in detail.

It has been shown that the number of fungal species and colonies in a profile falls off with depth, the rate of reduction being dependent on the depth of the soil and the nature of the soil horizons. Most species occur most abundantly in the surface layers of the soil. A few species were commoner in subsurface layers, and a few were abundant near the chalky boulder clay of the C horizons. Fungi show different distributions in the five soils, and two large groups may be distinguished: those common in the acid soils and those common in the alkaline soils. Species of *Penicillium* were particularly abundant in the acid soils.

Microscopic examination of soil has shown that fungi are present both as spores and mycelium. Study of incubated soil plates stained with lactophenol cotton blue has shown that most of the colonies develop from humus particles. Viable spores have also been found in these soils, especially the acid ones.

INTRODUCTION

From the numerous studies on soil fungi there has gradually grown up the concept of a fungal flora of the soil, a flora which, although showing variation in different countries and different soils, is sufficiently distinct to warrant recognition (Burgess, 1939). Speaking of this flora Waksman (1944) remarked that fungi form a very large and heterogeneous group of micro-organisms permeating the surface layer of the soil with their mycelium, their number and types gradually decreasing in the lower surface layers. Some fungi are widely distributed in soil and others are limited to certain habitats. The distribution of these organisms is influenced by the abundance and nature of the organic matter of the soil, as well as by other soil and climatic conditions, surface vegetation and soil management.

While the general nature of the soil flora has become recognized, details, particularly of the ecology of its members, are not always clear. All investigators agree that fungi are most abundant near the surface of the soil and that their number decreases with depth. It is recognized that some species of fungi may occur at considerable depths in the soil. Rathbun (1918) found *Fusarium* spp. and other fungi to a depth of 38 in.; and Waksman (1916) found *Zygorrhynchus vuillemini* at 12-20 in., and often as the only

organism in subsoils. However, as Timonin (1935) pointed out, there have been few studies on the distribution of organisms throughout the horizons of soil profiles. Gray and McMaster (1933), Gray and Taylor (1935) and Gray (1935) have studied the distribution of organisms in podsoils in Canada, and have shown that the organic horizons are biologically more active than the leached horizons, as measured by carbon dioxide production, rate of nitrification and the production of ammonia from urea. Timonin (1935) studied virgin podsol soils in Canada and showed that total numbers of organisms were greatest in the surface horizons and lowest in the subsoil. The biological soil horizons generally conformed with the pedological horizons. With fungi isolated from the soils studied by Timonin (1935), Bisby, Timonin and James (1935) found that there was no striking difference in the fungus flora of the different horizons. Nevertheless, they concluded that certain species may be characteristic of certain types of horizons of soil.

There are considerable differences of opinion regarding the species of fungi found in different soils, which, considering the diversity of soils, is not surprising. Werkenthin (1916) and Brierley (1928) found no difference between the fungi from cultivated and uncultivated soils. Jensen (1931) reported *Penicillia* and *Trichoderma* common in acid soils and *Mycogone nigra* (Morgan) Jensen and *Coccospora agricola* Goddard common in alkaline soils. It is generally agreed that soils of northern latitudes are characterized by an abundance of *Mucorales* and *Penicillia*, while *Aspergilli* are predominant in warmer soils. The genus *Trichoderma* is found especially in acid and in waterlogged soils. With other organisms there is less agreement. Hagem in 1910 isolated *Mucorales* from the soil of meadows, gardens and forests, but observed that they are most abundant in forest soils. Jensen (1931) found *Mucorales* to be abundant in field and garden soil. Campbell (1938) found more species of *Mucorales* in cultivated soil than in moor and peat-bog soils, but concluded there was no specific distribution of the *Mucorales* with different soil types.

Many of the papers on the distribution of fungi in soil have dealt with species in agricultural soils, and less is known about the occurrence of fungi under natural soil conditions. The question of an ecological distribution of fungi in such soils has received little attention. Ling-Young (1930) pointed out that in order to obtain a fair idea of an endemic microflora, soils should be examined from such localities as forests, peat-bogs and mountains which remain untouched by man.

The present work was undertaken to investigate the influence of soil type and soil horizon on the distribution of fungi in some natural grassland soils.

THE SOILS STUDIED

The soils studied in this investigation were the grasslands A, B, C, D and E described by Watt (1936, 1940) at Lakenheath Warren, Suffolk. These soils have been derived from chalky boulder clay and interglacial sands, the underlying formation being chalk. The soils are uniformly sandy, but show considerable variation chemically; where the chalk is near the surface the calcium carbonate content and pH value are high, but in other areas

the base status and pH value are low and deep well-developed podsoles are present. Grasslands A and B are calcareous and alkaline, grassland C varies about neutrality, and grasslands D and E are highly acid at the surface. In general the soils are dry, poor, and unsuitable for agriculture. According to tradition Lakenheath Warren has never been cultivated, but there is some evidence that parts may have been cultivated in early mediaeval times. Fig. 1 shows average profiles of these soils.

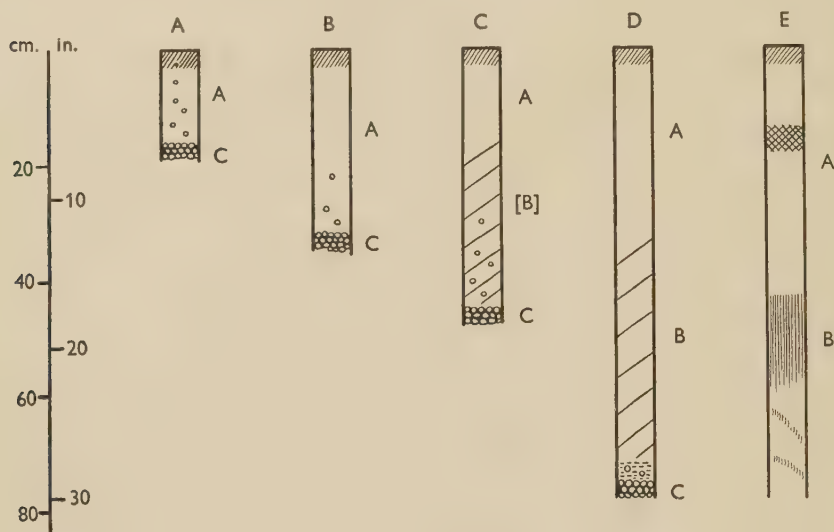


Fig. 1. Profiles of the grassland soils studied; for description see below.

Descriptions of the soil profiles

Grassland	Soil horizon	Depth in in.	pH	Description
A	A	0-7.5	7.8	Grey with numerous chalk stones
	C	7.5-	8.4	Compact chalky boulder clay
B	A	0-6	7.6	Medium dark sand
		6-13	8.0	Lighter with numerous chalk stones, the number increasing with depth
C	C	13-	8.4	Compact chalky boulder clay
	A	0-5	6.4	Dark sand coloured by humus
	(B)	5-11	7.0	Reddish brown sand
		11-18	7.6	Numerous chalk stones in an earthy matrix
D	C	18-	8.0	Compact chalky boulder clay
	A	0-12	4.4	Pale grey sand
	B	12-28	6.0	Light yellow sand
		28-30	6.8	Dark red-brown sticky layer
E	C	30-	8.0	Chalky boulder clay: surface very uneven, deep pockets of sand penetrating the boulder clay
	A	0-5.5	3.8	Ash-grey sand
		5.5-7	4.0	Dark, humus-coloured sand
		7-18	4.2	Ash-grey sand, usually lighter than near the surface
	B	18-	4.6	The uppermost 2.5 in. is a dark purplish brown humus-iron pan. Portions of this project downwards as inverted cones with yellow stratified sand in between

COLLECTION OF SOIL SAMPLES AND ISOLATION OF FUNGI

Soil samples were collected throughout the profile of each soil type. A soil profile was exposed by digging a pit about 9 in. square, and deep enough to include part of the C horizon of the soil. This depth varied from sample to sample and soil to soil; however, few samples were collected below 30 in. One face of the pit was prepared as the profile and small sterilized collecting tubes, 2 x 1 in., were pushed horizontally into the face at successive 1 or 2 in. intervals. Samples were collected every inch to 8 in. and thereafter every second inch. Early experiments showed that the upper layers had a greater variety of fungi than the lower layers of the profile.

The isolation plates were prepared by transferring a small amount of soil, about 0.005-0.015 g., from a collecting tube into a sterilized Petri dish, 8-10 ml. of cooled medium was added and the soil particles dispersed throughout the agar. With most soil samples an adequate dispersal was obtained by shaking and rotating the plate before the agar solidified. If, however, the soil was very dry, it was preferable to mix the particles with a drop of sterile water in the plate before the medium was added. Dox agar + 0.5% yeast extract, acidified to pH 4.0 by the addition of phosphoric acid, was used as the isolation medium. This type of isolation plate with a fine layer of soil particles distributed throughout an agar film has been called a soil plate (Warcup, 1950).

Soil plates were incubated at 22.5° C. and examined at intervals during a month for the isolation or recording of fungi. Some species were rarely recorded unless the isolation plates were kept for a considerable period of time. The examination was carried out by scanning the plates with a wide-field binocular microscope (mult. 20). This instrument allows a good recognition of the colony habit of fungi. Known fungi were recorded without isolation, but all doubtful or unknown colonies were isolated. Preliminary studies had indicated the colony characteristics of many of the commonly occurring species. Several groups of fungi, such as *Mortierella*, *Mucor* and *Pythium*, had always to be isolated, as species could not be distinguished by colony characteristics alone. An effort was made to isolate and record all the sporing fungi present and as many as possible of the non-sporing species. Notes were taken of the occurrence of any other organisms present.

A few supplementary methods were tried for the isolation of fungi from Lakenheath Warren. Water and hemp seed (Butler, 1907) for the isolation of water moulds was tested on several occasions in both winter and summer. Species of *Mortierella* and *Pythium* were isolated. Members of the Saprolegniaceae and allied water moulds do not seem to be present in the dry soils of Lakenheath Warren. Plates of sterilized rabbit dung and soil were prepared for the growth of Ascomycetes, but no Ascomycetes other than *Chaetomium funicola* were found by this method. Plates prepared with grassland A soil often developed the fruit bodies of *Dictyostelium mucoroides*, a member of the Acrasieae.

NOTES ON THE GROUPS OF FUNGI

In an investigation of this nature it is impossible to examine in detail all the fungal colonies which grow on the soil plates or to isolate more than a few of them. At least 200,000 colonies grew on the isolation plates, and about 2000 of these were isolated for further study. The common procedure was to examine several cultures of a fungus from the same or preferably different soils and then keep a 'representative' culture which was given a culture number. It was the representative culture which was identified. Usually further specimens were isolated at a later date and compared with the culture kept in the collection. Where no name has been given to a fungus, it is referred to throughout this paper by its collection number. Herbarium specimens of many of the cultures were kept, and some specimens or cultures were deposited at the Commonwealth Mycological Institute. These are indicated by an asterisk in the list of species. The writer wishes to acknowledge with thanks the kind assistance of those mycologists mentioned below, who have examined many cultures and helped with the identification of certain groups of fungi.

Phycomycetes were common in the soils of Lakenheath Warren and were represented by thirty-six species. Three species belonged to the Oomycetes, the rest were Zygomycetes. Miss G. M. Waterhouse, of the Commonwealth Mycological Institute, Kew, kindly examined the species of *Pythium*. Zygomycetes were classified according to Zycha (1935) except for the genus *Mortierella*, for which the monograph by Linnemann (1941) was used. Species of *Mortierella* were the most common Phycomycetes found, both by number of colonies and by number of species. Some species did not spore readily on Dox + yeast agar, but would do so on weaker media such as soil agar.

A method employed for the rapid examination of many species of *Mortierella* was to grow the fungi on water. A piece of agar containing the fungus was carefully placed on the surface of sterilized water in a Petri dish. Many species grew out from the floating agar and spored as they travelled across the surface of the water. This method was also used to decide the approximate taxonomic position of sterile phycomycetous cultures. All Oomycetes examined grew in the water, while all Zygomycetes (mainly species of *Mortierella*) grew out over the surface of the water.

The genera *Absidia*, *Mucor* and *Zygorrhynchus* were also common. It was found that zygospores of heterothallic species of Mucorales were often encountered on soil plates, contrary to the general opinion that the two strains of heterothallic species of these fungi rarely occur together in nature. Zygospores were common in three of the four heterothallic species of *Absidia*, and were also obtained in three of the six heterothallic species of *Mucor* and the species of *Cunninghamella*.

Several parasitic Mucorales were isolated. One of these, a species of *Syncephalis* growing upon *Mortierella* spp., was of wide occurrence and very common. It was probably even commoner than recorded, since the formation of sporangiophores of the parasite appeared to be partly dependent upon the medium of the isolation plates. On a weak medium such as soil agar the parasite usually fruited vigorously; on Dox + yeast

agar it did not always spore but was present as a mass of fine yellow hyphae over the *Mortierella* species.

Though Bisby, James and Timonin (1933) have recorded that Ascomycetes, producing asci in culture, are rarely found in soil isolations, twenty-four species were recorded from the soils of Lakenheath Warren. Eleven of these, including most of the common species, belonged to the Plectomycetes of Gwynne-Vaughan and Barnes (1937). Common genera were *Thielavia* and *Gymnoascus*. Yeasts were occasionally seen but were not critically examined. No Discomycetes were isolated. Dr S. J. Hughes, of the Commonwealth Mycological Institute, Kew, kindly examined the *Chaetomium* species and some of the other Pyrenomycetes. Many of the Ascomycetes, although growing well upon soil plates, would not grow or spore satisfactorily on Dox + yeast agar in pure culture. Other media had to be employed, and in a few cases no medium was found on which the fungus grew as abundantly as on the soil plates. Lochhead and Chase (1943) have reported several types of soil bacteria which require unidentified substances in soil extract for growth.

Several Basidiomycetes were also isolated from the soils of Lakenheath Warren, but the occurrence of these fungi in soil has been discussed elsewhere (Warcup, 1951).

Most of the species isolated from the soils of Lakenheath Warren were Fungi Imperfecti. Species of *Penicillium* were common, particularly in the acid soils. Dr K. B. Raper kindly named the representative cultures sent to him. *Fusaria* were common and were identified by Dr W. L. Gordon of the Dominion Laboratory of Plant Pathology, Fort Garry, Manitoba. *Gliocladium* species and a species of *Cylindrocarpum* were also common. *Aspergilli* were rare and were identified from the manual by Thom and Raper (1945). Dr M. B. Ellis, of the Commonwealth Mycological Institute, Kew, kindly identified the species of *Papularia* and a culture of *Periconia*. *Alternaria* and *Stemphylium* were rare and were identified from the monograph by Neergaard (1945).

A few of the fungi which occurred on the soil plates were neglected, since they were known to be present in the air of the laboratory as contaminants. Under this heading come several species of *Penicillium*, *Botrytis cinerea* and *Cladosporium herbarum*. However, *C. herbarum* was so frequent on the soil plates that it is probable that it occurs in soil.

In examining large numbers of isolations from soil one inevitably fails to determine every fungus. A number of the fungi seen on the soil plates were not obtained in pure culture. Many of these were non-sporing, darker-coloured Hyphomycetes. Identifications were not obtained for 34 of the 148 species which were isolated and given collection numbers. Two cultures formed only sclerotia, four only chlamydospores and eight remained sterile on all media tested. Cultures have been kept of most of the unknown sporing species, and further work may enable them to be identified.

The following list of species gives the names of the fungi isolated, with a query and collection number where there is any doubt as to its identity.

LIST OF SPECIES

Specimens or cultures deposited at the Commonwealth Mycological Institute are indicated by an asterisk.

PHYCOMYCETES

Oomycetes

- Pythium acanthicum* (?) Drechsler (B135)*
P. aphanidermatum (?) (Edson) Fitzpatrick (B83)*
Pythium sp. (B120)

Zygomycetes

- Absidia butleri* Lendner*
A. cylindrospora Hagem (+, -)
A. glauca Hagem (+, -)*
A. orchidis (Vuillemin) Hagem (+, -)
Actinomucor repens Schostakowitsch
Circinella rigida G. Smith
Coemansia erecta Bainier
C. pectinata Bainier
Cunninghamella sp. (+, -)

- Mucor dispersus* Hagem (+, -)
M. hiemalis Wehm. (+, -)
M. lausannensis Lendner
M. racemosus Fres.
M. ramannianus Möll.
Mucor sp. (+, -) (B102)
Mortierella alpina Peyronel
M. bainieri Costantin
M. dichotoma (?) Linnemann (B61)
M. humilis Linnemann
M. isabellina Oudemans
M. minutissima van Tieghem
M. sepedonioides Linnemann
M. vinacea Dixon-Stewart
Mortierella spp.
Syncephalis sp. (B55)
Zygorrhynchus moelleri Vuill.
Z. vuillemini Namyslowski

ASCOMYCETES

Plectomycetes

- Arachniotus* sp. (B65a)
Aspergillus nidulans (Eidam) Wint.*
Gymnoascus subumbrinus A. L. Smith & Ramsb.*
G. setosus (?) Eidam (B40)*
Gymnoascus sp. (B60)
Penicillium luteum Zukal*
Thielavia sp. (B3)
Thielavia sp. (B16)

Pyrenomycetes

- Cainea verruculosa* (Jens.) Hughes ined. (B84)*
Chaetomium funicola Cooke*
C. olivaceum Cooke & Ellis*
C. trilaterale (?) Chivers (B104)*
C. sp. (B72)
Chaetomium sp. (B155a)*
 (?) *Melanospora* sp. (B68)*
 (?) *Perisporium vulgare*? Corda (B137)*
Sordaria fimicola (Rob.) Ces. de Not.*
S. setosa Wint.*

FUNGI IMPERFECTI

- Acrostalagmus cinnabarinus* Corda*
Aleurisma carnis (Brooks & Hansf.) Bisby
Alternaria tenuis auct.
Aspergillus fumigatus Fres.*
A. nidulans (Eidam) Wint.*
A. terreus Thom
A. ustus (Bainier) Thom & Church*
A. versicolor (Vuill.) Tiraboschi*
A. versicolor series (B23)
Cephalosporium spp.
Cladosporium herbarum Link
Cylindrocarpon radicola Wollenw.
Fusarium oxysporum sensu Snyder & Hansen*
F. sambucinum Fuckel

- F. scirpi* var. *acuminatum* (Ellis & Everh.) Wollenw.*
Gliocladium catenulatum (?) Gilman & Abbott (B128)
G. fimbriatum Gilman & Abbott
G. roseum (Link) Bainier
Gliocladium sp.
Gliomastix convoluta var. *felina* (Marchal) Mason
Helminthosporium sp.
Humicola grisea Traaen
Monilia geophila Oudemans
Monotospora sp. (B18)
Myrothecium sp. (B12)
Oidiodendron fuscum Robak
Paeecilomyces sp. (B5)

FUNGI IMPERFECTI (cont.)

- Paecilomyces* sp. (B 54)
Papularia arundinis (Corda) Fr.
P. sphaerosperma (Pers.) von Höhnelt*
Penicillium aculeatum Raper & Fennell*
P. canescens Sopp*
P. fellutanum Biourge*
P. frequentans Westling*
P. janthinellum Biourge*
P. lilacinum Thom*
P. nigricans (Bainier) Thom*
P. piscarium Westling*
P. pulvillorum Turfitt*
P. stoloniferum Thom*
P. terlikowskii Zaleski*
P. duclauxi (?) Delacroix (B43)*
P. islandicum (?) Sopp (B14)*
P. purpurescens (?) (Sopp) Raper & Thom (B129)*
P. roseo-purpureum (?) Dierckx (B94)*
P. rugulosum (?) Thom (B103)
- P. janthinellum* series (B138)
P. namyslowskii series (B8)
P. raistrickii series (B15)
Penicillium sp. (B13)
Periconia macrospinosus Lefebvre & A. G. Johnson*
Pestalotia sp. (B105)
Phoma glomerata (Corda) Wr. & Hochl.
Phoma sp. (B27)
Pullularia pullulans (de Bary & Low) Berkhout
Spicaria violacea Abbott
Stachybotrys atra Corda*
Stemphylium consortiale (Thüm.) Groves & Skolko
Stysanus sp. (B2)
Trichocladium asperum (Cda.) Harz.
Trichoderma viride Pers. ex Fr.
Volutella ciliata Fr.

THE METHOD OF RECORDING THE FUNGI

Soil samples were collected at intervals over a period of 14 months, from April 1947 to May 1948, in order to obtain data on the constancy and place of occurrence of the microfungi in the soils of Lakenheath Warren. During that period twenty samples were taken from each of the five soils. Supplementary samples have been taken and confirm the original data, but these results are not recorded in detail.

Fungal species were recorded against the depth and the region of the profile in which they occurred. Each soil sample consisted of the tubes of soil collected at intervals down the profile. Three soil plates were prepared from each tube of soil and were treated as a unit, and a fungus was given a positive record if it occurred on one or more of the three plates. This allowed, as far as possible, for such factors as competitive growth, the active suppression of one fungus by another, or the chance absence of a fungus from any particular isolation plate. *Trichoderma viride* often suppressed many of the colonies which were developing on a soil plate. A great development of *Pythium*, *Mortierella* and other fast-growing fungi made it difficult to record slower growing species. The absence of a fungus from one or more of the soil plates was not, of itself, always clear evidence that the fungus did not occur at that level in the soil, since there is not a completely uniform distribution of fungi throughout the particles of a soil. In most samples, however, the common fungi occurred on all three plates from the same depth.

In soil samples collected at monthly intervals, there was little evidence of seasonal fluctuations in the occurrence of most fungi. The variation in fungal populations from samples collected a few yards apart on the same date was as great as that recorded from samples taken over a period of time. This gave all twenty samples an equal value and allowed the results to be used to plot the frequency of occurrence of fungi in the soil. Since it was

evident that occurrence was correlated with soil horizon rather than with depth in inches, the data from each sample were correlated with the average profile of a soil. This compensated for the considerable variation in depth at which the horizons of a profile occurred; for instance, in grassland D the depth of the chalky boulder clay varied from 24 to 48 in.

Since the number of species isolated was very large, only the more common fungi have been considered in detail. A common fungus was one which occurred in at least ten of the twenty samples from any soil. This figure, chosen to divide the species into common and rarer fungi, was arbitrary and without special significance.

NUMBERS OF SPECIES OF FUNGI

Each of the five soils at Lakenheath Warren was found to have a distinct population of fungi. These populations differed in numbers of species and in specific composition. The number of species recorded from a profile, and the total number of species obtained, differed from soil to soil. There was variation in the constancy of occurrence of fungi and in the number of common fungi which were isolated from a soil. A general analysis of the numbers of fungal species isolated from the five soils is given in Table 1.

Table 1. *Numbers of species isolated from twenty profiles of each of five soils*

Soil	...	A	B	C	D	E
Total no. of species		48	62	74	79	69
Av. no. of species per profile		23	33	37	35	25
No. of species from only one profile		6	11	12	17	17
No. of common species		21	27	33	31	21

Table 1 shows that the highest number of species occurred in grassland D, the soil with the widest range of structure from highly acid surface sand to alkaline chalky boulder clay. The acid soils differ from the alkaline soils in having a higher number of species which were isolated only once. Grassland C has the richest fungal flora, if a high number of species and a high number of common fungi may be said to indicate a rich flora. Grasslands B and D are not greatly different from C, but the extreme soils of the series, the highly alkaline A and the highly acid E, have poorer floras, particularly with respect to the frequency of occurrence of fungi. These results, indicating a large and varied flora in alkaline as well as in acid soils, are interesting with regard to the generally accepted view that alkaline soils have a limited fungal flora.

Since this view is based on counts of fungal numbers, a count of the number of colonies per gram was made on samples of surface soil from each of the five grasslands. Soil samples were collected at 3 in. deep and ten isolation plates prepared from each sample by the dilution technique (Waksman, 1927). The dilution was 1:5000, the medium acidified Dox + yeast agar. The plates were counted after 3 days. Plates from grassland E soil were so crowded with colonies that an estimate rather than a count was made for this soil. Table 2 records the number of colonies per gram of soil and the pH of the samples. In these samples the higher the acidity the greater was the number of colonies per gram of soil.

Table 2. *Numbers of colonies of fungi in five soils*

Numbers in thousands per gram of soil					
Soil ...	A	B	C	D	E
pH of sample	8.0	7.5	6.4	4.2	3.9
No. of colonies	36	59	74	245	425

For comparison Table 3 records the average number of species which were isolated by the soil-plate method from the 3 in. level in twenty profile samples of each of the five soils. Table 3 shows that the alkaline-slightly acid grasslands B and C contained the greatest number of species of fungi. Thus although the acid soils showed a greater number of colonies per gram than the alkaline-slightly acid soils, the high numbers in the former were produced by fewer species of fungi. Examination of the isolation plates from grasslands D and E showed that the high fungal counts for these acid soils were largely due to colonies of *Penicillium*. Microscopical examination of samples of E soil showed that many of these colonies developed from spores which were present in the soil. It would seem that in these acid grasslands a high number of colonies per gram may reflect a high sporing capacity of a few species of fungi rather than a high level of fungal activity in the soil.

Table 3. *Numbers of species of fungi in five soils*

Soil ...	A	B	C	D	E
No. of species	11	17	18	12	10

VERTICAL DISTRIBUTION OF FUNGI

The vertical distribution of fungi in the five grasslands, obtained by plotting the average number of species recorded from each depth against the soil profile, is given in Fig. 2.

There was a marked falling off in the number of species as one sampled deeper in a soil. In grasslands A, B and C, the rate of reduction appeared to be dependent on the depth of soil above the chalky boulder clay. Grassland D showed a slight rise in the number of species at 3-6 in., a rapid fall in the remainder of the A horizon, and relatively constant numbers throughout the B and C horizons. In grassland E the number of species fell off gradually with depth. The data for this grassland, however, were difficult to correlate as some profiles lacked or had a poorly developed humus-iron pan in the B horizon, and in these profiles fungi extended deeper into the soil.

There was a reduction in the number of colonies, as well as in the number of species as one sampled further down a profile. Soil plates prepared from samples near the surface of a profile often showed as many as 100 colonies, while those from deeper samples might show five or less. Few colonies developed on plates prepared from chalky boulder clay, and occasionally plates from other deep samples remained sterile or developed only bacterial or Actinomycete colonies. This was particularly the case in the B horizon of the E soil, and plates prepared from samples taken below the humus-iron pan often remained sterile.

Few fungi appeared to be strictly limited to any particular horizon of one or more of the soils. However, it was often difficult to distinguish accurately where one horizon stopped and the next commenced. There may also be some transference of fungi as spores by water movements or some other agency through the soil (Burgess, 1950). Single colonies of a fungus sometimes occurred in regions of a soil where that particular organism was not usually found. Most species showed maximum occurrence at or near the surface of a soil, though there were a few striking exceptions.

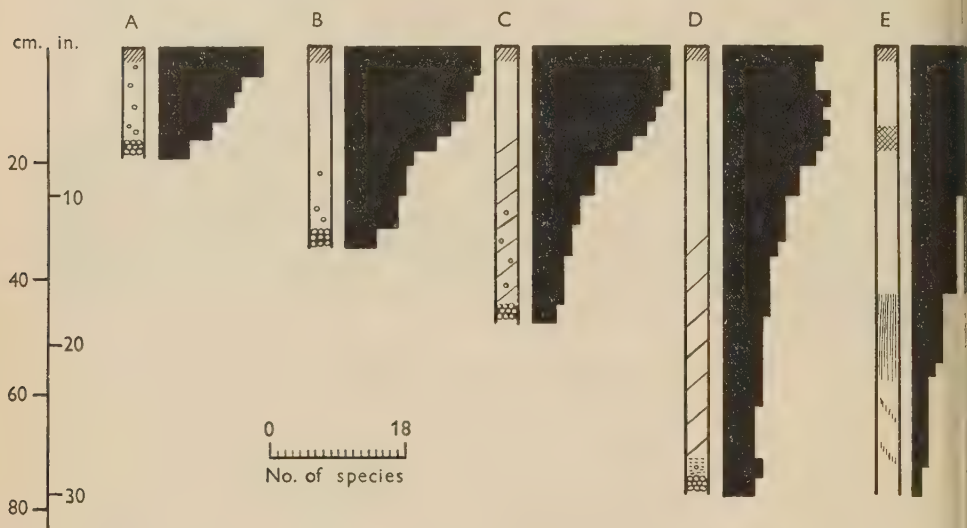


Fig. 2. Average distribution of species in the grassland soils.

A few species, *Absidia glauca*, *A. orchidis* and *Mucor lausannensis*, were confined to the surface humus regions. A few, *Gymnoascus* B6o, *Cylindrocarpon rademicola* and *Paecilomyces* B5, showed maximum occurrence near the lower part of the A horizons and were rarely recorded near the surface of the soil. *Mortierella alpina*, *M. minutissima* and *Spicaria violacea* showed maximum occurrence in or near the chalky boulder clay. Only the two *Mortierella* species were found at all consistently in the chalky boulder clay and then mainly near the surface of the layer. *M. minutissima*, however, has been isolated 8 in. below the surface of the chalky boulder clay in grassland A.

Many of the data on the distribution of fungi throughout the profile are hard to evaluate, as some of the fungi which appear so common in the lower horizons are slow growing and may be present in the surface regions, though rarely appearing on the crowded isolation plates from those samples.

DISTRIBUTION OF SPECIES IN FIVE SOILS

The fungal populations of the five grassland soils differed in composition as well as in numbers. Table 4 shows the distribution of the common species of fungi in these soils. This table records only occurrence in a soil, and gives no indication how abundantly a species occurred throughout a profile.

Table 4. Occurrence of common fungi in twenty samples of each of five soils

	Soil				
	A	B	C	D	E
B124	10	.	.	.	.
Humicola grisea	14	2	.	.	.
B32 (sterile culture)	20	1	.	.	.
Fusarium sambucinum	20	5	4	.	.
Thielavia B3	20	17	1	.	.
Myrothecium B12	19	16	16	.	.
Monotospora B18	15	20	18	.	.
Cephalosporium B46	14	19	17	.	.
Gliocladium fimbriatum	1	19	7	.	.
Absidia glauca	.	20	16	.	.
Penicillium luteum	.	12	11	.	.
Penicillium B13	.	15	17	.	.
Paecilomyces B54	.	3	14	.	.
Pythium B120	10	1	4	1	.
Syncephalis B55	12	6	5	1	.
Mortierella alpina	20	20	20	15	.
Phoma B27	18	20	20	5	.
B132	18	19	11	2	.
Penicillium lilacinum	4	19	18	5	.
Stysanus B2	2	20	20	1	.
B64 (sterile culture)	1	19	19	7	.
Mucor lausannensis	13	14	18	1	.
Mortierella (dichotoma) B61	4	2	10	3	.
Fusarium scirpi var. acuminatum	3	7	14	1	.
Gymnoascus subumbrinus	.	8	10	5	.
Thielavia B16	.	19	20	7	.
B75	.	9	19	16	.
Spicaria violacea	20	.	1	2	1
Mortierella minutissima	20	20	20	11	3
Gliomastix convoluta	20	20	20	20	3
Penicillium nigricans	20	20	20	20	18
Fusarium oxysporum	19	20	20	14	4
Cylindrocarpon radiculicola	11	19	19	18	3
Cephalosporium B45	5	20	20	20	15
Trichoderma viride	9	10	14	20	20
Zygorrhynchus vuillemini	1	4	17	20	20
Penicillium fellutanum	.	20	20	17	2
Absidia cylindrospora	.	20	20	16	7
Penicillium (raistrickii series) B15	.	9	15	20	8
Paecilomyces B5	.	17	17	20	10
Mortierella B88	.	4	17	20	19
Penicillium canescens	.	9	7	15	13
Penicillium (namyslowskii series) B8	.	.	6	11	17
Trichocladium asperum	.	.	1	19	16
Mortierella humilis	.	.	.	10	2
Absidia orchidis	.	.	.	20	9
Penicillium pulvillum	.	.	.	18	9
Penicillium janthinellum	.	.	.	16	4
Gymnoascus B60	.	.	.	14	6
Melanospora B68	.	.	.	13	10
B90 (sterile culture)	.	.	.	11	13
Mortierella isabellina	.	.	.	5	10
Penicillium frequentans	.	.	.	5	13
Mortierella B26	.	.	.	5	16
Oidiodendron fuscum	.	.	.	7	16
B1	.	.	.	14	16
Mucor (ramannianus group)	.	.	.	15	20
Penicillium piscarium	.	.	.	16	20
Penicillium (islandicum) B14	.	.	.	17	20
Mortierella vinacea	.	.	.	20	20
Penicillium terlikowskii	.	.	.	20	20

An interesting feature of Table 4 is that, with one exception, none of the common fungi were confined to any single soil type. This was perhaps to be expected, as each of these 'soil types' contained within itself wide variations in structure which would give diverse fungi a chance of growing therein. Soils B and C, which were similar, had a very similar range of fungi occurring within them. Likewise the A horizons of soils D and E contained many fungi in common.

In the five soils studied there are two large groups of fungi, that of the acid soils and that of the alkaline soils. Many species were confined to one of these two groups, others, though more widespread, showed greater occurrence in either the acid or alkaline range of soils. Jensen (1931) also noted that certain species of fungi were abundant in alkaline soils and others were abundant in acid soils. The observations of Jensen and myself suggest that soil fungi live in ecological communities which are governed by factors such as soil type. It is not known whether the pH of the soil is the dominant factor which determines fungal communities of the type indicated here. The terms 'acid' and 'alkaline' have been used as convenient labels by which to describe differences in the soils studied.

MICROSCOPIC EXAMINATION OF SOIL

Microscopic examination of soil was undertaken as a check and a guide to the interpretation of the results obtained from the study of soil plates. The fact that fungi could be isolated from samples of chalky boulder clay or from samples of soil collected at considerable depths indicated their presence, but gave no indication if they were active in such regions of the soil. It was hoped that direct examination of soil would elucidate this.

Two methods were used, soil was either stained directly on a slide, or first dispersed in agar (Jones & Mollison, 1948). In the second method soil plates were prepared with more soil than usual and using a cleared Difco agar. Commercial agar contains algal particles which absorb stains and make the microscopic examination of soil very difficult. Plates were either stained immediately, in which case plain agar was used, or first incubated for 12-48 hr., in which case Dox + yeast agar was used. The plates were treated as follows: The surface of the agar was flooded for 5 min. with lactophenol cotton blue diluted in water (1 ml. in 10 ml.); after this brief staining the agar surface was rinsed with distilled water, drained, and the plates stored for a day or two before examination. The stain gradually accumulated in fungal threads, spores, bacteria, actinomycetes, and some of the humus particles. Plates were either examined directly, or strips of agar and soil particles were cut out and examined on a slide. In this way it was possible to trace the origin of many of the colonies which were developing on the soil plates. Information on the identification of these fungi was gained by removing hyphal tips and growing them before staining the plates.

Samples from different horizons of the grassland soils were examined by the methods outlined. Fungal hyphae and spores were found in all the samples examined, from surface samples to samples of chalky boulder clay collected at a depth of 28 in. in grassland D. In surface samples fungi were

present as both dead and viable mycelium and dead and viable spores. Much of the fungal mycelium was in very close contact with the soil particles, including the large quartz grains. Thom (1927) and others have remarked on the intimate association between fungal hyphae and soil particles.

Much of the mycelium which wandered from soil particle to soil particle appeared dead, as it lacked cell contents and did not grow on the incubated plates. The samples thus studied were collected during the summer when soil moisture was low. Most of the fungal colonies that grew on the incubated plates developed from humus particles, the fungus often appearing to come from inside the particle. It was usually impossible to judge whether the fungus was present as a spore or as mycelium in a humus particle. Mycelium free and capable of further growth (in the agar) was difficult to find, but was occasionally seen. Experiments with a copiously mycelial soil fungus, *Psalliota arvensis*, have also shown how little free mycelium capable of further growth occurred on dilution plates even after shaking the suspension for half an hour. Twenty dilution plates (1:500), prepared from grassland soil containing the mycelium of *P. arvensis*, were stained with lactophenol cotton blue and the source of the basidiomycete colonies traced. The results are given in Table 5.

Table 5. *Source of colonies of Psalliota arvensis on dilution plates*

Source of colony	Free mycelium	Humus particle
No. of colonies	14	406

The fact that most fungal colonies on an isolation plate develop from humus particles probably explains the striking drop in the number of colonies and species of fungi in the B horizons of the grassland soils, since these horizons are deficient in humus.

Spores were present, particularly in the surface layers of the acid soils. Many, including spores of *Penicillium*, *Mortierella*, *Zygorrhynchus* and *Trichosporium*, proved capable of germination on the incubated plates.

IMPORTANT ORGANISMS IN THE GRASSLAND SOILS

Many of the organisms isolated from Lakenheath Warren have been shown to have a limited distribution in these soils and in the horizons of their profiles. An analysis of the occurrence of the more important genera and species will now be given.

Micro-organisms other than fungi

Nematodes, although rare, were recorded from grasslands A, B and C, and were found on soil plates from as deep as 14 in. in the C soil. They occasionally flourished on a soil plate, and by spreading bacteria and spores over the agar surface made the recording of fungi very difficult. Only general observations were made on the occurrence of the soil bacteria. The medium used was made highly acid to keep down bacterial numbers, but even under these conditions some bacteria grew on plates from all the soils. They were rare and sporadic in the highly acid E soil,

most common in the alkaline A soil, and frequent in the other soils. Their greatest development was on plates from the A horizon and from the partly leached chalky boulder clay at the surface of the C horizon.

Dictyostelium mucoroides Bref. was recorded from grasslands A and B. This organism was common throughout the top 0-7.5 in. of grassland A soil but was absent from the chalky boulder clay; it was rarely found in grassland B and then only near the surface. It was not obtained from any of the other soils. Singh (1947) found *Dictyostelium* spp. from only three of twenty-nine samples of grassland soils and found no correlation between distribution and pH of the soil in soils ranging from pH 4.3-7.8. *D. mucoroides* occasionally developed on the soil plates, where sorocarps appeared after 2 weeks.

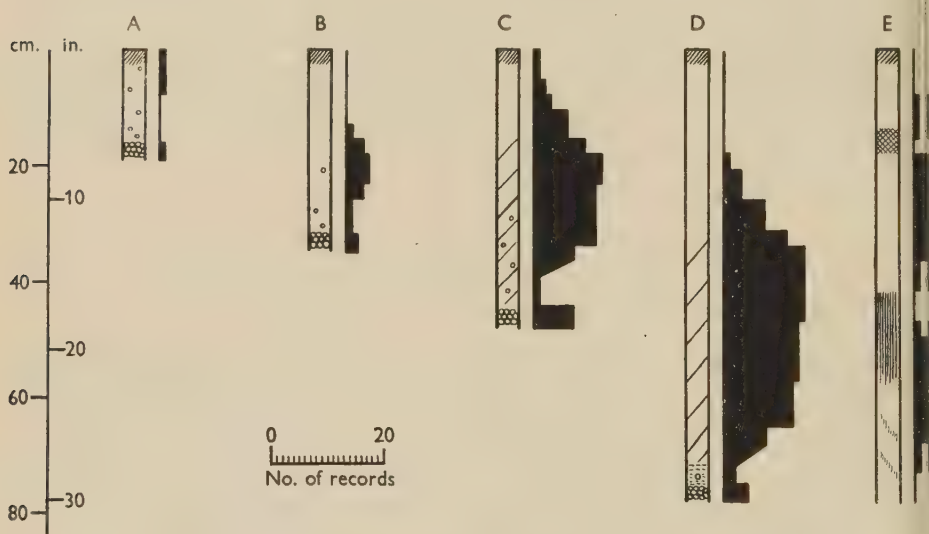


Fig. 3. Regions of high Actinomycete numbers in the grassland soils.

Actinomycete colonies normally developed on most of the plates from a soil profile. Some plates from soils C and D showed a great development of Actinomycete colonies, and occasionally they were the only organisms appearing on the soil plates. These high concentrations also tended to occur in different samples of the same regions of these soils. Fig. 3 shows the profile regions in which Actinomycete colonies were noted in especially high numbers.

High numbers of Actinomycete colonies were recorded mainly from the B horizons of grasslands C and D, the regions of these soils with low humus and low moisture content. They occurred at considerable depth, and were relatively infrequent near the surface.

Phycomycetes

Three species of *Pythium* were isolated from the grassland soils, but two were rarely obtained. *Pythium* B120 was unusual in being one of the few

fungi which showed a seasonal distribution. It was regularly isolated during winter months when soil moisture was relatively high, but was not recorded at other seasons of the year.

Mortierella proved to be the most common Phycomycete genus in the grassland soils. The number of species isolated was large (second only to species of *Penicillium*), and some species were abundant throughout the soil profiles. Disby *et al.* (1935) also found members of this genus common in the soils of Manitoba, and Chesters (1948) has noted the importance of species of *Mortierella* in soil near Nottingham.

The *Mortierella* species were found to fall into two groups: species in the first group were common in the alkaline soils and rare in the acid soils (or occurred near the chalky boulder clay in grassland D); species in the second group were more strictly confined to the acid soils. Linnemann (1941) noted that different *Mortierella* species occur in different soils, and has given the pH values at which many of her species were found. Table 6 records the range of grassland soils from which the species of *Mortierella* were isolated, their pH range and the pH range given by Linnemann.

Table 6. Occurrence of *Mortierella* in five soils

Species	Soils	pH	
		Lakenheath	Linnemann
<i>M. alpina</i>	A B C D	8.6-6.0	7.6-6.5
<i>M. (dichotoma) B 61</i>	A B C D		
<i>M. minutissima</i>	A B C D E	8.6-4.6	7.8-6.0 (5.3)
<i>M. B 88</i>	B C D E		
<i>M. B 61 a</i>	C D		
<i>M. vinacea</i>	D E	6.0-3.8	6.5 & 5.0
<i>M. isabellina</i>	D E	4.5-3.8	4.2-3.8
<i>M. B 26</i>	D E		
<i>M. bainieri</i>	D E		
<i>M. humilis</i>	D E	6.0-3.8	6.2-3.7
<i>M. sepedonioides</i>	E	4.0-3.8	4.7

M. alpina was common near the chalky boulder clay in the grassland soils. It occurred throughout soil A, where it was recorded from all samples studied. In soils B and C the fungus was still very common throughout the profile, but in soil D it was found predominantly near the chalky boulder clay at the bottom of the profile. *M. alpina* was absent from three out of four profiles of the D soil, where the chalky boulder clay was not reached at a depth of 30 in. (Fig. 4).

Four species of *Absidia* were found, three of which were common. All species were heterothallic, and with three species zygospores were often found on the soil plates. *A. glauca* in grasslands B and C, and *A. orchidis* in grasslands D and E, were both sharply defined in situation, occurring in the surface 0-4 in. of soil. They were also common in the litter zone. Dale (1912, 1914) isolated *A. glauca* from chalky soil and *A. orchidis* from sandy soil in England. *A. cylindrospora* was a very common fungus and more widespread throughout the soil profiles than the other two species. It was not isolated from soil A, was common throughout the profiles of soils B and C, and rarer and almost restricted to the A horizons of soils D and E. Campbell (1938) recorded *A. cylindrospora* as abundant in soils in Scotland.

Mucor species were common in the A horizons, rare in the B horizons and absent from the C horizons of the grassland soils. Most species were confined to the surface 0-4 in. layer of soil and were more often isolated from the raw humus of the litter layer than from the mineral layers. An

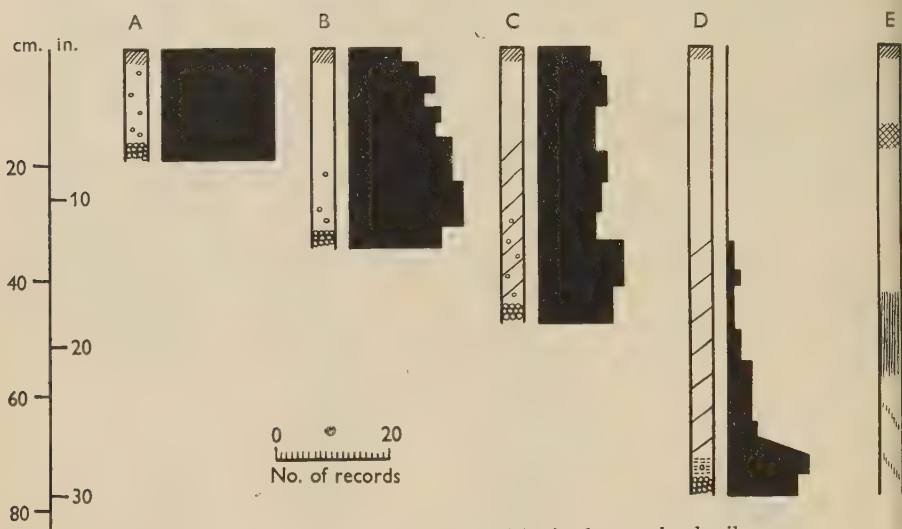


Fig. 4. Occurrence of *Mortierella alpina* in the grassland soils.

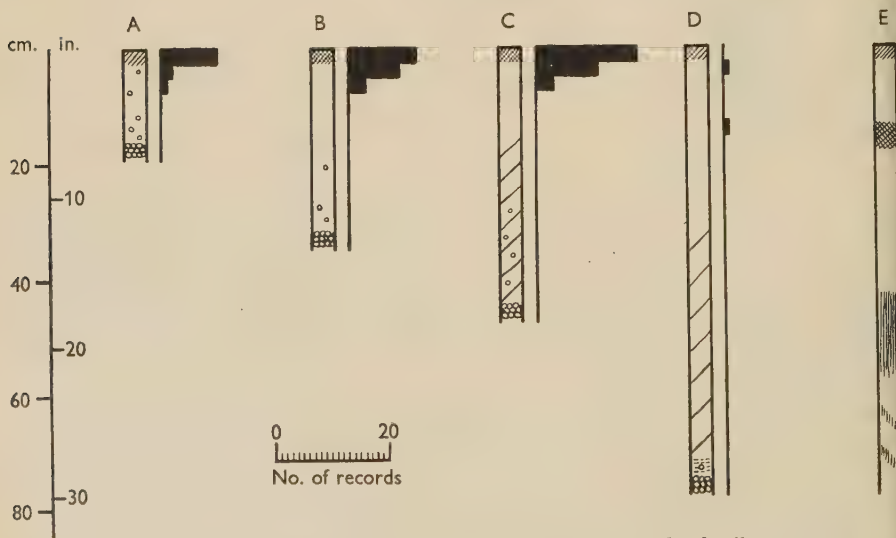


Fig. 5. Occurrence of *Mucor lausannensis* in the grassland soils.

exception was a strain of *M. ramannianus* which was confined to grassland E and was common in the B and lower part of the A horizon and rarely recorded from surface samples. Although more species of *Mucor* were found than species of *Absidia* or *Zygorrhynchus*, the latter genera were more widely distributed throughout the soil profiles and gave higher numbers of

colonies per plate. Fig. 5 plots the occurrence of *Mucor lausannensis* in the profile of the grassland soils. *M. lausannensis* has also been isolated from chalk and peat soils by Dale (1914).

Zygorrhynchus vuillemini was common and occurred predominantly in the acid D and E soils. In these soils it was common in the A horizons and rarer in the B horizons; in the alkaline soils it occurred only in the upper layers of the A horizons. The distribution of *Z. vuillemini* in these grasslands is noteworthy as Waksman (1917) and others have recorded this species as common in subsoils.

Ascomycetes

While several of the species isolated from soil were rare, and apparently occur more frequently on other substrata, dung, etc., others were so common in the soil that they must be considered as soil inhabitants.

Three species of *Gymnoascus* were obtained and two of them were common. *G. subumbrinus* was isolated from soils B, C and D, and was rare near the surface, absent from the chalky boulder clay, and sporadic throughout the remainder of the profiles. It is a very slow-growing fungus which usually

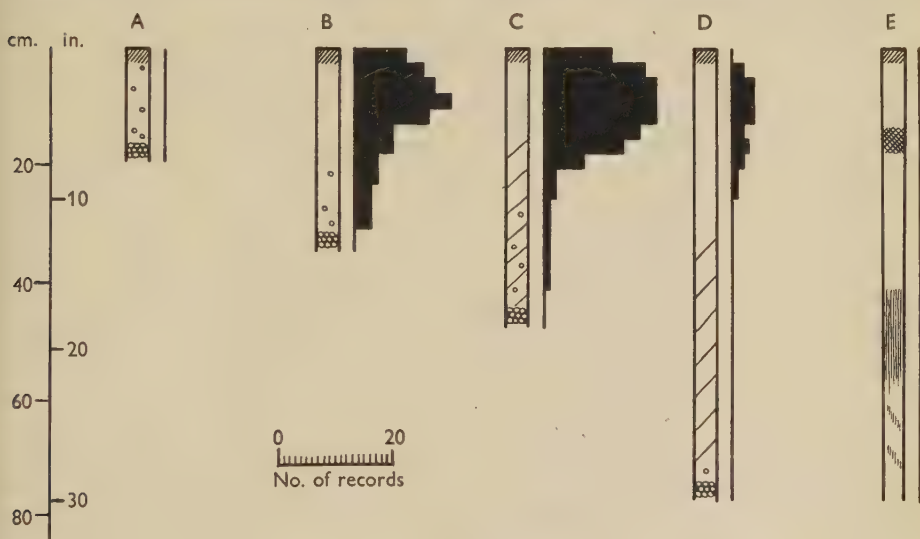


Fig. 6. Occurrence of *Thielavia* B16 in the grassland soils.

appeared as a single colony on a soil plate, and which may have been more abundant in the soils than recorded. *Gymnoascus* B60 was isolated mainly from grassland D, where it occurred in the lower part of the A horizon and throughout the B horizon. It was not found in the chalky boulder clay. Both these species of *Gymnoascus* seem to prefer the drier regions of the grassland soils.

Two species of *Thielavia* were common in the alkaline soils and Fig. 6 shows the distribution of *Thielavia* B16 in these soils.

Species of Pyrenomycetes were rare and usually of rather sporadic occurrence in the grassland soils. Culture B68, a *Melanospora* species, was

the most common Pyrenomycete. It was frequent in the surface layers of grasslands D and E, where it was usually isolated from a depth of 1 in. and occasionally from as deep as 6 in. Several species of *Chaetomium* were found but were rare. They usually occurred in the surface layers of the soil.

Fungi Imperfecti

The majority of the colonies and species of fungi isolated belonged to the Fungi Imperfecti. *Penicillium* constituted the most important genus and a large number of species were isolated. Most soil mycologists have recorded species of *Penicillium* as common in North Temperate soils. In the grassland soils most of the species of *Penicillium* were found in the very acid soils and they were comparatively rare in the alkaline soils. Jensen (1931) noted that *Penicillia* were common in acid soils, while Bisby *et al.* (1935) found evidence that some species of *Penicillium* were characteristic of certain types or horizons of soil. Brian, Hemming and McGowan (1945) have noted the prevalence of species of *Penicillium* in the acid soils of Wareham Heath. Several of the species from Wareham Heath are also found in the acid soils of Lakenheath Warren. Table 7 shows the soils from which the species of *Penicillium* were isolated.

Table 7. Occurrence of *Penicillium* in five soils

Species	Soil
<i>P. (rugulosum)</i> B103	A
<i>P. luteum</i>	B C
<i>P. B13</i>	B C
<i>P. (roseo-purpureum)</i> B94	C
<i>P. lilacinum</i>	A B C D
<i>P. nigricans</i>	A B C D E
<i>P. stoloniferum</i>	A B C D E
<i>P. canescens</i>	B C D E
<i>P. fellutanum</i>	B C D E
<i>P. (raistrickii series)</i> B15	B C D E
<i>P. (duclauxi)</i> B43	C D E
<i>P. (namyslowskii series)</i> B8	C D E
<i>P. aculeatum</i>	D E
<i>P. frequentans</i>	D E
<i>P. (islandicum)</i> B14	D E
<i>P. janthinellum</i>	D E
<i>P. piscarium</i>	D E
<i>P. pulvillorum</i>	D E
<i>P. (purpurescens)</i> B129	D E
<i>P. terlikowskii</i>	D E
<i>P. (janthinellum series)</i> B138	E

Most of the species of *Penicillium* were found predominantly in the A horizons of these soils. A few, such as *P. canescens*, *P. roseo-purpureum*(?) and *Penicillium* B15, were restricted to the surface layers of the soil. On the other hand, *P. lilacinum* was rarely obtained from surface samples but occurred deeper in the A horizons. *P. canescens* occurred as two slightly different strains, one common in soils B and C and the other in soils D and E. Smith (1946) reports that this species is very common in English soils. *P. islandicum*(?) was common in the acid soils D and E, where it was rare in the surface layers, and common in the lower layers of the A horizons and the upper layers of the B horizons. *P. duclauxi*(?) was unusual in occurring

mainly in deep samples and was recorded from as deep as 36 in. in grassland E soil. *P. pulvillorum*, which was frequently isolated from the A horizons of the acid grasslands, was originally isolated from a very acid clay in Middlesex (Turfitt, 1939). *P. nigricans* was the commonest fungus recorded from the grassland soils. In the A soil it was common in the surface layers, in soil B it was common throughout the whole profile, in soils C and D it was common in the A horizons but rarer in the B horizons, and in soil E it was mainly found in the surface layers of the soil.

Several species of *Aspergillus* were found, but all were rare and very sporadic in occurrence. *A. nidulans* occurred occasionally in surface samples from soil C; and *A. versicolor* was found occasionally in all the soils, occurring more frequently in the deeper soil samples.

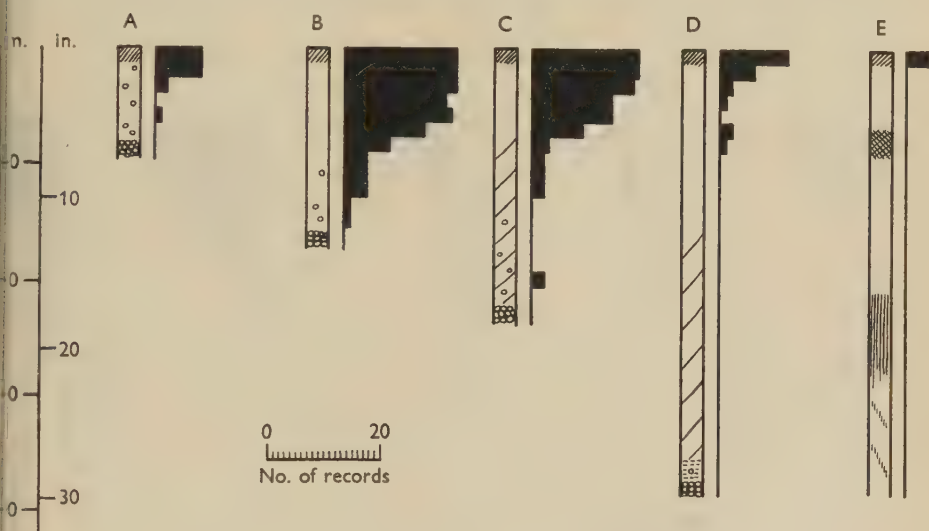


Fig. 7. Occurrence of *Fusarium oxysporum* in the grassland soils.

Cylindrocarpon rademicola occurred in all five soils and was common in soils B and C and in the B horizon of soil D. The number of colonies per plate was greater from samples with high moisture content than from those with low moisture content.

Three species of *Fusarium* were isolated from the grassland soils and are species which are common in grassland soils throughout the world. *F. oxysporum*, represented by several strains, was isolated from all five soils. Fig. 7 plots the distribution of this fungus in the profiles of the five soils.

F. sambucinum was common in the surface layers of soils A, B and C, and *F. scirpi* var. *acuminatum* occurred in the surface layers of soils A, B, C and D.

Several species of *Gliocladium* were found. *G. fimbriatum* was the commonest species and occurred in the A horizons of grasslands A, B and C. It was rare in the A soil, common in the surface layers of the B soil and rarer in the C soil.

Gliomastix convoluta was abundant in the grassland soils. It was usually slow to develop, but after 3 or 4 weeks the soil plates were often covered by the trailing hyphae and black spore heads of this fungus. Mason (1941) records that this species has often been isolated from English soils.

Trichoderma viride was common in the grassland soils, particularly in the A horizons of the acid soils D and E. It was much rarer in the alkaline soils and usually occurred near the surface of these soils. Bisby (1939) noted that *T. viride* was rather uncommon in some alkaline soils, and Rishbeth (1949), while investigating *Fomes annosus* root-rot of pines, found *Trichoderma viride* to be far more abundant in acid than in alkaline soils in East Anglia. A *Gliocladium*-like strain was occasionally found in the B soil and greatly resembled the strains of *Trichoderma viride* discussed by Brian (1944). Microscopic examination showed *T. viride* was present both as spores and mycelium in samples of grassland E soil. Fig. 8 plots the distribution of this fungus in the profiles of the five soils.

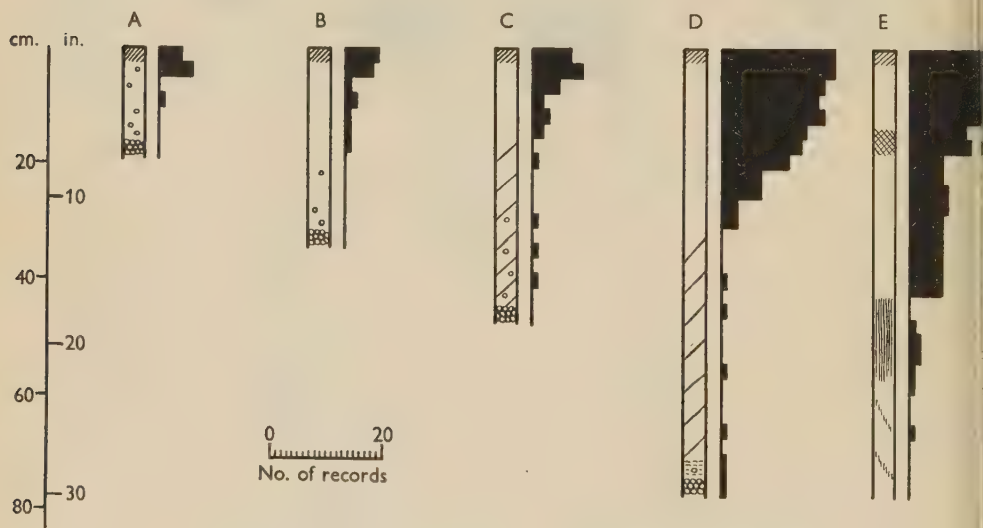


Fig. 8. Occurrence of *Trichoderma viride* in the grassland soils.

DISCUSSION

The investigations on the vertical and horizontal distribution of fungi, recorded in this paper, allow one to formulate a general picture of the fungal microflora of the grassland soils of Lakenheath Warren. It is, of necessity, a limited picture, as the work has been of a general nature, and many questions remain untouched.

Microscopic examination of soil has shown that fungi are present both as spores and as mycelium. Mycelium has been found traversing the spaces between soil particles and closely adherent to their surface. Much of the free mycelium, however, lacked cell contents and appeared to be dead. On soil plates most fungal colonies were found to develop from humus particles or spores rather than from pieces of mycelium. Thom (1927) has

remarked that active fungi in soil must be conceived of as a series of inter-lacing networks of hyphae composing the mycelia of the various species. It would seem, however, that in the dry, sandy soils of Lakenheath Warren, fungi are present predominantly in humus particles or as spores, and that they only become active and spread under moister conditions or with additional nutrient.

Fungal spores were found predominantly in the surface-soil layers, and less frequently in the deeper samples. Most spores appeared to be viable, though a few were seen which lacked cell contents. Little information was obtained on their formation, the length of their survival in soil, or their movement through soil. Burges (1950) has found that the 'wet' spores of *Glomastix* were washed down through soil more readily than the 'dry' spores of *Penicillium*. That spores may be washed down in natural soils is indicated, since surface fungi were occasionally found as single colonies much deeper in the soil.

As most of the colonies on soil plates developed from humus particles it is probable that low humus content is a limiting factor for fungal growth in the deeper layers of the grassland soils. In the B horizons of grasslands C and D the soil is light yellow in colour, and Watt (1940) has shown that these layers contain less than 0.5% carbon. The lack of energy sources would explain the great reduction in the number of colonies and species in the B horizons of these soils. Other factors, however, may be involved, since Timonin (1935) and others have shown that the number of micro-organisms in peat decrease with depth, despite a high humus content throughout. A few species were regularly found in the deeper soil layers, and some of these have very low carbon requirements as judged by their growth in culture. Some such as *Mortierella* spp. were found to fruit better on water agar or other very weak media than in the presence of sugars. Newman and Norman (1941, 1943) have shown a clear difference in activity between surface and subsurface populations. The ability of the surface population to produce carbon dioxide from cornstalks mixed into the soil was shown to be greater than that of the subsurface population.

It has been found that many species of fungi may be repeatedly isolated from a soil over a period of several years. About a third to a half of the fungi obtained from a grassland soil were found in half or more of the profile samples collected, and several species were found to be always present. In samples collected at monthly intervals there was little evidence of the influence of environmental factors on the fungal populations. It is thought, however, that samples collected more frequently may give evidence on these matters.

Studies of the five grassland soils have also shown that different fungal populations occur in different soils, and that the distribution of soil fungi is influenced by soil type. The fungi in these soils may be divided into two main groups: those occurring in the alkaline soils, and those in the highly acid soils. Many species were confined to one of these two groups, others, though more widespread, showed greater abundance in either the acid or alkaline range of soils. Jensen (1931) also noted that certain species were abundant in alkaline soils and others were abundant in acid soils.

These investigations on the occurrence of fungi in soil indicate that many

methods are necessary to gain an understanding of a soil population. A count of the number of colonies on dilution plates showed that the acid soils had a high number of fungi per gram of soil, whereas soil plates indicated that relatively few species were present and that these were mainly *Penicillia*. Microscopic examination of the acid soils showed the presence of many fungal spores. It seems, therefore, that a count of the number of colonies in these soils does not necessarily indicate a high degree of fungal activity, but rather the occurrence of conditions favourable to spore production of a few species of fungi, or the development of species that are profuse spore-producers. Jensen (1934), Chesters (1948) and others have stressed the fact that different methods of soil microbiology are complementary rather than mutually exclusive.

This investigation was carried out during the tenure of a Postgraduate Research Scholarship of the University of New Zealand. I wish to express my thanks to Prof. F. T. Brooks, F.R.S., under whose supervision this work was carried out; to Dr A. S. Watt for advice on the soils of Lakenheath Warren; and to Dr P. W. Brian for supplying some cultures of soil fungi.

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ON ROZELLOPSIS SEPTIGENA

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(With Plate 18 and 2 Text-figures)

Rozellopsis septigena (Fischer) Karling, is reported from *Saprolegnia* sp. Infection of host, sporangium development and zoospore liberation are described. It was found possible to infect a pure culture of the host and, to a lesser extent, *Saprolegnia delicata*. The parasite could not be induced to attack *S. ferax* and *Achlya* sp. The systematic status and position of *Rozellopsis septigena* remain obscure.

INFECTION, SPORANGIAL DEVELOPMENT AND LIBERATION OF ZOOSPORES

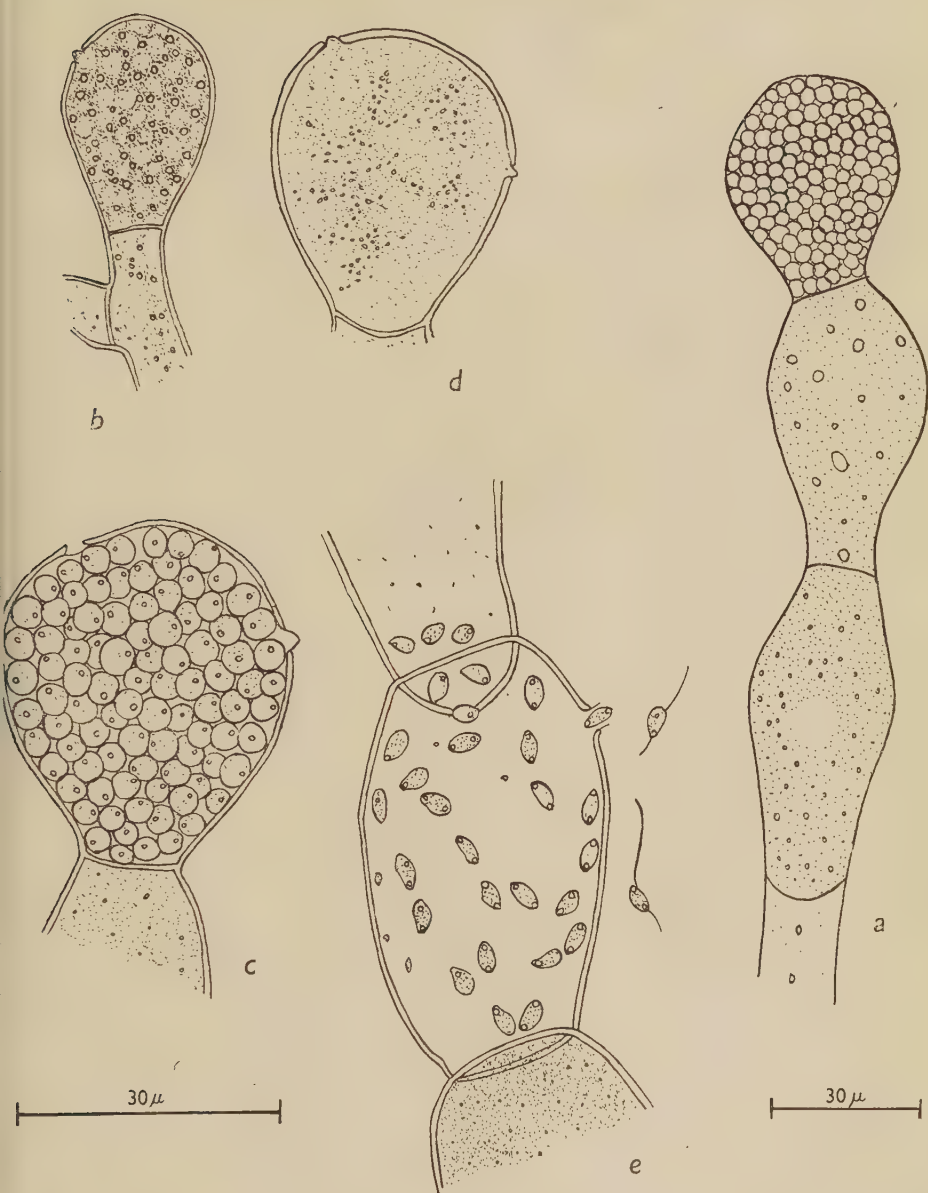
Rozellopsis septigena, a parasite of *Saprolegnia* causing marked hypertrophy of the hyphae, was found in a collection from a pond at Riverhead, Sevenoaks, Kent. A species of *Saprolegnia* was growing on dead mosquitoes, and the parasitized hyphae had a beaded appearance, so that they showed a strong resemblance to the fertile hyphae of *Allomyces* (Text-fig. 1a). It became clear that the parasite belonged to the genus *Rozellopsis*, and, since so little is known of members of this genus and since *R. septigena* has not previously been recorded from Britain, a careful study of the organism was made (Pl. 18, figs. 1, 2).

The biflagellate zoospores are pyriform, hyaline, $3 \times 5-6 \mu$, heterokont, with two, or sometimes four, minute refractive granules. The shorter flagellum is about 5μ long, the longer 10μ long (Text-fig. 2a). Staining by Löffler's method (Couch, 1938) indicated that the long flagellum is clearly of the whip-lash type. This staining was much less successful with the short flagellum, but there were indications that it is of the tinsel type.

The zoospores were observed to swim with a very even motion, quite unlike the jerky movement of the larger zoospores of the host. The shorter flagellum, which is directed forwards during swimming, was seen to undergo slow spiral movements, while the long flagellum apparently trailed passively behind. When the long flagellum touched a host hypha, it became attached to the surface as previously described by Fischer (1882). The short flagellum then disappeared, whilst the zoospore was drawn smoothly down to rest on the surface of the host hypha by the contraction of the long flagellum. This process was observed on several occasions.

The zoospore normally encysts and germinates on the surface of the host and open communication is established between them. Then refractive oil globules in the host protoplasm stream away from the point of infection. The protoplasm in the host around the point of infection becomes denser, and this denser region is at first delimited by a clear peripheral zone. Soon no limiting zone is to be seen, but the region of dense protoplasm spreads considerably from the point of infection (Text-fig. 2). Two examples were

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Text-fig. 1. Formation of zoosporangia of *Rozellopsis septigena* Karling, in infected hyphae of *Saprolegnia*. a, infected hypha showing the formation of linear series of zoosporangia of the parasite. b-d, zoosporangia showing dehiscence papillae. e, emergence of zoospores. Scale on right refers to a, that on left to c-e.



Text-fig. 2. Early infection of *Saprolegnia* sp. by zoospores of *Rozellopsis septigena*. a, biflagellate zoospores of the parasite. b-g, infected hyphae showing the dense appearance of the protoplasm in the region of infection and the oil droplets streaming away from the point of infection. c and g also show the clear zones near the infected region in the early stages. f, germination of a zoospore outside the host, producing a short prolongation (‘germ-tube’?).

observed where zoospores settled on the host surface and induced the characteristic streaming of oil globules and spreading opacity in the host protoplasm; this opacity continued to spread, despite the fact that the zoospores had swum away without penetrating the host wall. These observations suggest that the streaming of oil globules and the development of denser protoplasm in the host are initiated by some substance produced by the zoospore. After penetration, the zoospore case remains attached to the host wall, but is not completely vacated by the protoplasm of the parasite until the opacity in the host protoplasm has spread considerably (Text-fig. 2*d*). At no stage can the protoplasm of the parasite be clearly distinguished from that of the host, at least using the usual methods of staining. If a plasmodium exists it must be spread throughout the host protoplasm in an extremely fine condition. On the other hand, in the Plasmodiophorales the infecting organism enters as single, clearly observable, naked masses of protoplasm and no zoospore case is left attached outside.

Several hours after infection, the whole of the protoplasm in the infected hyphae has become opaque and tends to concentrate in terminal swellings which develop in these same hyphae. Large vacuoles appear in a linear series, in basipetal succession, and cross-walls are laid down between the vacuoles, apparently by the host, producing the characteristic chain of sporangia. The terminal sporangium becomes swollen and ripens first, the others following in succession. The protoplasm in a sporangium becomes very opaque and then small vacuoles appear, gradually coalescing into a central one. By this time the parasite has a membrane clearly demonstrable by plasmolysis with sugar solution. The protoplasm in the sporangium then takes on an appearance as if forming cleavage furrows, but this never progresses far and the protoplasm suddenly becomes clear and apparently homogeneous. It is at this stage that the exit papilla of the zoosporangium first becomes evident as a bulge in the wall of the swelling which contains the zoosporangium. The clear stage may persist for as long as 24 hr. but eventually the protoplasm takes on a foamy appearance, with vacuoles of even size and equally spaced (Text-fig. 1*b*). This condition persists for about half-an-hour, when the protoplasm again becomes clear. Then, after 1 hr. or so, it becomes reticulate and cleavage into zoospore initials takes place. During this stage, or even earlier, the protuberance of the host wall deliquesces and only the delicate papilla of the sporangium protrudes through the host wall (Text-fig. 1*d*). Within the sporangium the zoospores begin to swirl round in a characteristic manner until the dehiscence papilla breaks down, and then they emerge singly and swim away (Text-fig. 1*e*). Waterhouse (1940) has recorded a similar series of changes in the protoplasm during the maturation of the zoosporangium in *R. waterhousei* Karling, and in both the species the liberation of zoospores is strikingly similar.

Resting spores were recorded by Fischer, but although the material was kept under observation for several months, no such spores were observed in this culture.

CULTURAL EXPERIMENTS

Rozellopsis septigena was found on an unidentified species of *Saprolegnia*, and accordingly the host was brought into pure culture on oatmeal agar. It was found that a small piece of this culture material, when transferred to sterile water for 24 hr., could be very heavily infected by introducing a hypha of the original host material bearing a ripe zoosporangium of the parasite. Repeated inoculations were made from this artificially infected material on to fresh host hyphae, always, with success. It was on the inoculated material that so many of the early stages of infection were so clearly seen.

Similar attempts to transfer the parasite to pure cultures of *Saprolegnia ferax* and of an unidentified species of *Achlya* were quite unsuccessful and no sign of infection could be seen, despite repeated attempts. With *Saprolegnia delica* only a few infections occurred after very many attempts, and these did not develop very far.

So far as they go these observations confirm the findings of Fischer that *Rozellopsis septigena* is confined to species of *Saprolegnia*. *Rozellopsis simulans* shows great similarity with *R. septigena*, but so far has been found only on *Achlya* species as reported independently by Tokunaga (1933), von Minden (1915), and Fischer (1882). Fischer maintained that *Rozellopsis septigena* was confined to *Saprolegnia* and *Rozellopsis simulans* found only on *Achlya*. Karling (1943) has disputed these findings on the grounds that there have been no cross-inoculation experiments, and suggests that there may be only one species. He has, however, brought forward no evidence in support of his criticism, and it seems probable that the older view is correct. It is possible, however, that the two are really physiological strains of the one species, differing only in their host range.

SYSTEMATIC POSITION

The genus *Rozellopsis* was erected by Karling (1943) to include those *Rozella*-like species with biflagellate zoospores. Of the four species, there are two in which the sporangia occur singly, namely, *Rozellopsis inflata* Karling (*Pleolpidium inflatum* Butler) parasitic on *Pythium intermedium*, and *Rozellopsis waterhousei* Karling, parasitic on *Phytophthora* sp.; in the other two species, *Rozellopsis septigena* (Fischer) Karling, parasitic on *Saprolegnia*, and *Rozellopsis simulans* (Fischer) Karling, parasitic on *Achlya*, the sporangia occur in chains because of septation of the infected host hypha.

Rozellopsis septigena and *R. simulans* would seem to be closely related. Tokunaga figures anterior attachment of the two flagella in *R. simulans*, whereas in *R. septigena* it is quite definitely lateral attachment. However, too much importance should not be attached to this apparent difference, as it is often difficult to observe the insertion of the flagella with accuracy. The similarities of these two species are well marked, but their relationship with the other two species is less obvious. *R. waterhousei* and *R. septigena* show a remarkable similarity in the developmental stages leading to zoospore formation, and it seems likely, therefore, that the two species are related. *R. inflata* differs from *R. waterhousei* in that the zoospores are reniform, not



pyriform, and that *R. inflata* causes much more marked hypertrophy of the host. Apart from these differences the two species are very similar.

The true systematic position of *Rozellopsis* remains obscure. Karling (1943) places it in Woroninaceae, but it might almost equally well find a place in Olpidiopsidaceae, or perhaps better still be segregated in a separate family. A natural classification will be possible only when these simple holocarpic forms with biflagellate zoospores have received more extensive study.

My thanks are due to Prof. Ingold, Birkbeck College, for the valuable advice he has given me.

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EXPLANATION OF PLATE 18

- Fig. 1. Hyphae of *Saprolegnia* sp. infected by *Rozellopsis septigena* Karling, showing the beaded appearance of the linear series of sporangia of the latter. ($\times 150$.)
Fig. 2. An infected hypha of *Saprolegnia* enlarged, showing two zoosporangia of *Rozellopsis septigena*. ($\times 700$.)

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REVIEWS

Physiology of Fungi. By LILIAN E. HAWKER. (London: University of London Press, 1950.) xvi + 360 pp., 38 text-figures, 5 plates. 21s. net.

It is probably a partial consequence of the unprecedented success of applied mycology during the last decade that there has been a steadily increasing interest in basic mycological research, particularly in the physiology of fungi. As a matter of fact, the vast number of papers being published at present in this field makes it difficult even for the specialist to follow its development. Distinctions between various branches of science are always artificial, but there seems now to be sufficient reason for treating the physiology of fungi as a special subject, distinct from general plant physiology and from general microbiology.

For some time it has been a desideratum of those physiologists working with fungi to obtain a modern review or digest of the essential results within their branch of science, written by a competent person active in this field. Such a review will also be welcomed by the great number of research workers in medicine and industry who, though lacking the ordinary biological training, need information on experimental mycology. This need is met only partially by the existing text-books of plant physiology, which deal mainly with higher plants. A certain amount of information may be obtained from books and reviews of the biochemistry of fungi, a number of which have been published during recent years. In these cases, however, the approach has been primarily that of an enzymologist or an organic chemist, and many important aspects of the physiology of fungi are not considered.

Thus, there is no doubt that the *Physiology of Fungi* by Dr Lilian E. Hawker will be received with great satisfaction by several different categories of readers. Her task has certainly not been an easy one, but it has been solved in an exemplary way. Dr Hawker is a trained plant physiologist, whose contributions in various fields of experimental mycology are well known. Contrary to most of her colleagues in this branch, however, she is familiar with her fungi in the field as well as in the laboratory. Thus, in many connexions she is able to refer to relevant ecological facts, which makes the reading particularly stimulating.

In the short introductory chapter, the author outlines the typical life cycle of fungi. This will prove valuable for many readers not familiar with the elementary morphological facts and terms in mycology. The following chapter deals with the chief methods of studying the growth of fungi in pure culture, together with a discussion of the different types of variation. The main part of the book is then devoted to the various aspects of pure physiology. The author has purposely avoided giving more than a superficial treatment of the biochemistry of fungi, 'mould syntheses', and similar things, a decision which is justified because of the large number of modern reviews available on these subjects. After a short chapter on factors influencing the survival and germination of spores, the author finally describes and discusses the various forms of interaction between fungi and other organisms. Both subject and style contribute to making this chapter particularly interesting.

In a book such as this, where a large amount of knowledge must be concentrated into a rather limited number of pages, the greatest difficulty lies in the selection of essential facts, so as to attain proper balance between the different chapters. On the whole, the author has solved this problem well; though perhaps she may have overemphasized 'sporulation' at the expense of pure 'nutrition'.

The examples and references are well chosen. In rapidly developing fields of science the older literature is often overlooked, and it is to the honour of Dr Hawker that she pays due regard to the achievements of the pioneers of experimental mycology. In a few cases, however, important recent contributions have been omitted, e.g., the investigation of Quispel (1945) on the physiology of lichen symbiosis, the study of Westergaard and Mitchell (1947) on perithecium formation in *Neurospora*, and a few modern papers about preservation of fungus cultures under mineral oil.

So far as the reviewer is able to judge, very few and relatively unimportant physiological phenomena have escaped consideration, e.g. the transportation problem in fungi, which admittedly has been surprisingly little studied. Of particular value are the tables summarizing temperature relations, pH-dependence, etc., in various fungi. The figures, most of which are original, are very instructive.

Of the relatively few misprints and *lapsus calami*, none seem to mislead the reader. A few of the Latin names are not quite correct, and *Russula roseolus* (p. 86) must be *Rhizogon roseolus*. The formula of pantothenic acid (p. 92) contains two errors, and the discussion of the effect of this growth factor seems also to include some inconsistencies.

The author's comprehensive list of cited literature, comprising not less than 55 pages with 835 references, will greatly facilitate the task of the reader who is interested in obtaining a more profound understanding of the various subjects treated in the book. Furthermore, a list of modern reviews and books is appended to each chapter.

To the reviewer's knowledge, Dr Hawker's book is the first of its kind. It will certainly be included in the library of every botanical and microbiological department, while its reasonable price also places it within the reach of students and research workers.

NILS FRIES

Manual of Bacterial Plant Diseases. By W. J. DOWSON. (London: A. and C. Black, 1949.) viii + 183 pp., 40 black and white plates, 18 maps. 16s.

The early emergence of the fungi as the dominant group of plant pathogens, and the establishment of a strong mycological tradition in plant pathology, have tended to obscure the small but important group of plant pathogenic bacteria. This tendency was strengthened by the chaotic state of bacterial taxonomy which had resulted from the preoccupation of the classical bacteriology with the activities of the bacteria, rather than the bacteria themselves. Few mycologists were prepared to exchange the relatively ordered taxonomy of the fungi for this, so that while the individual bacterial plant diseases have been dealt with from time to time as the necessity arose, it is only recently that the taxonomy of the causal organisms has been given serious consideration. The training of plant pathologists in the principles of bacteriology has suffered correspondingly.

Until the publication of this work under review there was no collective account of the plant bacterial diseases and the methods for their study later than Erwin Smith's *Introduction to Bacterial Diseases of Plants* in 1921, since when there have been considerable advances in knowledge and methods. For this reason alone, therefore, the publication of Dr Dowson's *Manual of Bacterial Plant Diseases* is an important event. This work, however, is far more than an up-to-date compendium of diseases and techniques, for in his long career devoted to the study of plant bacterial diseases, the author has given much consideration to the bacteria concerned, and has been mainly responsible for establishing their classification on a sound and enduring basis. His views and conclusions are embodied in this work, so that the bacteria themselves, rather than the diseases, constitute the central theme.

The book is in three parts. Part I deals with the subject generally, and includes a review of the position of the Gram-negative plant pathogens in the older classifications. The genera in these were founded on only one or two characteristics, usually morphological. These inadequate definitions resulted in inconsistencies, such as the inclusion within the same genus of bacteria with widely differing biochemical activities, while conversely, bacteria with obvious affinities were placed in different genera, e.g. Bergey's genus *Erwinia* into which the peritrichous pathogens were separated from the closely related coliforms on the basis of plant pathogenicity alone. The classification adopted by the author is the one originally proposed by him in 1939, after a detailed investigation of the morphology and physiology of the Gram-negative plant pathogens. Each of his three genera, the emended *Bacterium* Ehrenberg and *Pseudomonas* Migula, and the new genus *Xanthomonas* Dowson, were founded on several characteristics, and represented for the first time a grouping of these organisms according to their natural affinities. Of less enduring but more immediate interest to those concerned with the diagnoses of plant diseases, is the possibility of using the distinguishing biochemical activities of these genera for their easy identification. Thus, on the basis of the author's definition, a Gram-negative plant pathogenic bacterium which ferments salicin can be recognized as a *Bacterium* sp.

without resorting to the difficult technique of flagella staining. In Chapter II these Gram-negative genera are defined, and their principal growth and biochemical characteristics tabulated. The remainder of Part I deals with the general symptoms of bacterial diseases and the methods of isolation and inoculation of bacteria.

In Part II, 'Methods', the preparation, standardization and the use of media in the culture and identification of bacteria is comprehensively described, and an account is given of bacteriological and histological staining procedures.

Part III constitutes the main body of the book. Beginning with the Gram-positive genus *Corynebacterium*, the more important species of the four plant pathogenic genera are discussed, and the symptoms and etiology of the diseases they cause described. Each description concludes with a short list of the more important references. The identification of individual species is facilitated by the inclusion of tables showing their main biochemical reactions. A series of excellent photographs illustrating methods and symptoms are grouped at the end of the book, together with world maps showing the distribution of the more important diseases.

In his preface the author advocates a sound training of plant pathologists in the principles of bacteriology and the investigation of plant bacterial diseases. Dr Dowson's book admirably supplies an essential and long overdue instrument for this purpose. The many who will thus undoubtedly receive their introduction to bacteriology through it, may, however, regret the somewhat cursory treatment of the more fundamental issues in the early chapters, particularly as the author's exposition has that rare brand of lucidity which so few text-book writers seem to achieve.

The discussion on classification and nomenclature, for example, although excellent, is rather limited in its conception, and might profitably have included some account of the systematic position of the plant pathogenic species in relation to the bacteria as a whole. In this respect the genus *Corynebacterium* is particularly neglected. But this criticism detracts little from the value and importance of this book. In its preparation, Dr Dowson has made a notable contribution to the literature of bacteriology and plant pathology, and has done a service of inestimable value to those whose duty it is to investigate or diagnose the bacterial diseases of plants.

J. E. CROSSE

Recent Advances in the Study of Plant Viruses. By KENNETH M. SMITH. 2nd Ed. (London: J. and A. Churchill, 1951.) viii + 300 pp., 52 figures. £1. 2s. 6d.

This is a second edition of the book first published in 1933. It has been entirely rewritten; some chapters from the first edition have disappeared altogether, and new chapters on electron microscopy and serology have been added.

The title has become a little misleading, for the book is now really a text-book with sections on symptomatology, physiology of diseased plants, insect relations, methods of transmission, methods of purification, properties of purified preparations, estimation of particle size, electron microscopy, virus strains, serology, control of virus diseases and classification and nomenclature of viruses. Not all of the work included is 'recent' (ten years is a long time in a young science like virology!), and more than half of the references in the bibliographies, which are appended to each chapter, are to work published prior to 1940. Nor, indeed, is the book confined entirely to plant viruses—the two largest tables (pp. 196 and 202) deal with estimates of the sizes of particles of animal viruses and bacteriophages.

In a work of this scope there are, of course, points open to adverse criticism. Chapter VIII on the sizes of viruses and the methods employed in their estimation is disappointing. (Incidentally, does not the phrase 'size of a virus' involve an assumption at present unjustified? What meaning does 'size of a protein' or 'size of a salt' have?) This chapter is proportionately too long and, for one biologist at least, overloaded with mathematical formulae. The bibliography includes nearly 200 references—none of them later than 1943. One would have imagined that, for example, the work of Bawden and Pirie (1945), on the effect of method of preparation on aggregation, had a bearing on any discussion on the size of tobacco mosaic particles. (The work in question is discussed in Chapter IX,

but is not considered in relation to the effect of particle size on serological activity (p. 270) or on type of serological precipitate (p. 274.)

Space could also have been saved by omitting the details of superseded methods of purification of tobacco mosaic virus (Chapter vi). The saved space might have been used to mention, in the appropriate sections, work by McWhorter (1941) on intranuclear inclusions (p. 27), Kleczkowski (1949, 1950) on local lesions (pp. 33-40), Bennett (1944) and Cochran (1946) on seed transmission of Dodder latent mosaic and Cherry ringspot viruses respectively (p. 114), Sylvester (1949) on the effect of discontinuous feeding of aphids (Chapter iv) and so on.

These criticisms, however, are minor ones. Some of the chapters are admirable and the book as a whole is worthy of praise. The style is clear and readable and the book is well produced. The illustrations, many of which are photographs of virus crystals or electron micrographs, are of a high standard. Misprints are few. It is the only book in this field which will bear comparison with Bawden's *Plant Viruses and Virus Diseases*. (The new editions of both were apparently in press at the same time and are similar in scope.)

Recent Advances in the Study of Plant Viruses can be recommended to students and all others who are interested in the study of viruses and who desire a book which is only slightly less detailed but *much* less expensive than *Plant Viruses and Virus Diseases*. The author and publisher are to be congratulated on producing such a useful and handsome book at what is, for these days, a moderate price.

I.W.P.

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CONTENTS

	PAGE
Cultural studies on <i>Ascochyta rabiei</i> with special reference to zonation. By Abdul Hafiz. (With Plate 8)	259
A new species of Hyphomycete attacking the stem eelworm <i>Ditylenchus dipsaci</i> . By J. Basil Goodey. (With 1 Text-figure)	270
<i>Calothyrium pinastri</i> . By M. B. Ellis. (With 1 Text-figure)	273
<i>Helicodendron giganteum</i> n.sp. and other aerial-sporing Hyphomycetes of submerged dead leaves. By Janet I. Glen-Bott. (With Plate 9 and 4 Text-figures)	275
Zalewski's ' <i>Clathrospira spirifera</i> '. By Agathe L. van Beverwijk. (With Plates 10-12 and 10 Text-figures)	280
A Petri-dish moist chamber. By W. G. Keyworth. (With Plate 13)	291
A <i>Phytophthora</i> crown rot of Campanula. By Betty J. Legge. (With Plate 14 and 5 Text-figures)	293
Graminicolous Pyrenomycetes. I. The conidial stage of <i>Tubeufia helicomycetes</i> . By John Webster. (With 2 Text-figures)	304
Graminicolous Pyrenomycetes. II. The occurrence of the perfect stage of <i>Helminthosporium teres</i> in Britain. By John Webster. (With Plates 15 and 16, and 2 Text-figures)	309
Graminicolous Pyrenomycetes. III. The occurrence of <i>Microthyrium culmigenum</i> on grasses in Britain. By John Webster. (With Plate 17 and 1 Text-figure)	318
The ecology of predacious fungi. I. Preliminary survey. By C. L. Duddington	322
Albidin, an antibiotic red pigment from <i>Penicillium albidum</i> . By P. J. Curtis, H. G. Hemming and C. H. Unwin	332
A survey of heterothallism in the Ustilaginales. By H. L. K. Whitehouse	340
The susceptibility of strawberry varieties to red core. By C. J. Hickman and Mary P. English	356
The Basidiomycetes of Skokholm Island. VI. Observations on certain uncommon species and varieties. By A. F. Parker-Rhodes	360
The species of <i>Usnea</i> in Great Britain and Ireland. By W. Watson	368
The ecology of soil fungi. By J. H. Warcup. (With 8 Text-figures)	376
On <i>Rozellopsis septigena</i> . By G. A. Prowse. (With Plate 18 and 2 Text-figures)	400
Reviews	406

INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and subheadings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, Cambridge University Press, 200 Euston Road, London N.W. 1, price 2s. 6d.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1934.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give an adequate description and figure, and to designate the types as required by the *International Rules of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.